AcuSyst-*Maximizer*®
Operations Manual

BIOVEST INTERNATIONAL

8500 Evergreen Boulevard
Minneapolis, MN 55433-6000 USA

www.biovest.com
Telephone: (763) 786-0302
Toll-free in the U.S.: (800) 325-1112
After Hours: (763) 786-0302 (voice prompt)
Fax: (763) 786-0915
Email: accountservices@biovest.com

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Revision History

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This manual describes the Biovest International ACUSYST-*MAXIMIZER* (p/n 500011-XXX rev. M) instruments.

The revision history of this manual is shown below.

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Rev. F	3/10	Udated for new pH probe options
Rev. G	7/13	Updated for new GEX and conversion to PDF
Rev. H	3/14	Updated inoculation procedure's clamping instructions

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Glossary

Welcome to ACUSYST (**A**utomated **CU**lture **SYST**em) technology from Biovest International, the most advanced technology available in hollow fiber bioreactors. The ACUSYST-*MAXIMIZER* (MAXIMIZER) belongs to a product line that is directed toward the pilot plant scale of producing secreted biologicals from mammalian cells. Average antibody harvests from this instrument can reach up to 1000 mg/day. The perfused hollow fiber cultures in this instrument are maintained by microprocessor control.

ABOUT THIS MANUAL

This manual is designed to assist you in understanding and operating the MAXIMIZER. It is a guide to the operations and features of the system and provides guidelines for laboratory procedures sometimes necessary in the preparation or operation of the instrument. Complete information on the following tasks is included to aid your work from start to finish.

- how to assemble and inoculate the cultureware
- how to program the microprocessor for growth and harvest conditions
- how to maintain and support the culture
- how to dismantle the cultureware at the end of the run

The manual is organized into six sections, plus a **Glossary**, five appendices, and this introduction. The sections are organized in the order in which procedures or knowledge is necessary, although some cross-referencing may be required. Read completely through each procedure before starting. To ensure efficiency and reduce the risk of contaminating the system, make sure you have all necessary supplies before beginning the procedure.

Familiarize yourself with the basics of Biovest's hollow fiber design by reading Section 2, **System Theory**. Then, use Sections 3 and 4 to learn the components of the instrument and cultureware. The milestones table in Section 6 gives you an idea of how to plan for laboratory preparations and needs of the instrument. Use it to prevent unnecessary delays in setup and to adjust process controls before inoculation.

Throughout the text, key terms will be in **bold** type when they are introduced and defined — check the **Glossary** for definitions. Notes (designated as ***NOTES:***) are used to call attention to unique or important applications to which you should pay particular attention.

SERVICE AND SUPPORT

Contact Biovest International's Account Services department for:

- Technical Service
- Customer Service
- Field Service
- Sales Information

We are always willing to discuss your questions, suggestions, or problems with the ACUSYST-*MAXIMIZER* cell culture system.

Account Services is available weekdays from 8:00 – 5:00 Central Time (GMT-6). Our contact information is on the manual's cover page. After-hours telephone callers hear recorded instructions for emergency technical assistance. Keep a copy of our telephone number posted near the *MAXIMIZER* for quick reference!

Technical Service

Contact us with any question you have when operating the *MAXIMIZER* instrument or cultureware. Please be ready to provide as much detailed information as possible, including: instrument serial number, cultureware manufacturing lot number, printed or electronic run log, process alarms, chronology leading to the situation, relevant previous run experiences, etc.

Customer Service

Appendix B has ordering information for common items.

Field Service

Appendix F has important information about Calibration and Preventive Maintenance services that keep your *MAXIMIZER* operating optimally and consistently to ensure its highest production, lowest operating cost, and minimal unplanned downtime. We offer a Full Service Contract to simplify maintenance of the instrument over its many years of usage. If it is malfunctioning, we provide full repair services. *MAXIMIZER* is not user-servicable.

Sales Support

We're happy to help you purchase any of Biovest's products or services!

The ACUSYST-*MAXIMIZER* is a hollow fiber cell culture system. Both suspension and anchorage-dependent cells are grown in hollow fiber bioreactors which are maintained in the *MAXIMIZER*. The *MAXIMIZER* is designed to maintain cells in an optimal environment, one that mimics the mammalian body. In fact, the analogy of a body is used to describe how the system functions.

Cells are grown in the space outside of the fibers of a bioreactor. Media, the "blood" of the system, carry fresh nutrients and oxygen to the cells while carrying away cell wastes such as ammonia and CO_2. Media are circulated via the **circulation pump** acting as the "heart" of the *MAXIMIZER*. The "lungs" of the system, an oxygenator diffuses an air and CO_2 gas mixture into the media, oxygenating the media while maintaining the prescribed pH **setpoint**.

The microprocessor-based control system is the "brain" of the *MAXIMIZER*. It not only monitors but also provides feedback control to the various parts of the system, ensuring that environ-mental conditions are maintained in their operational ranges.

Cell-secreted product remains in a circuit separate from the waste products and may be harvested by activating a separate pump as required.

With these general concepts in mind, the following detailed description will give you an understanding of how and why the *MAXIMIZER* work.

HOLLOW FIBER TECHNOLOGY

The core of the *MAXIMIZER* mammalian cell culture systems is the hollow fiber **bioreactor (BRX)**. The BRX is a plastic cylinder containing several thousand **hollow fibers** attached at each end of the tube. Cells are grown in the space outside of the fibers known as the **extracapillary space**, or **ECS**. Growth media are circulated through the inside of the fibers, known as the **intracapillary space**, or **ICS** (Figure 2-1).

The fibers provide a substrate upon which the cells can grow. These fibers also permit the exchange of nutrients and wastes. The fibers of the BRX are semi-permeable and allow small molecular weight molecules, such as glucose and ammonia, to move freely across the fiber. The cells, growth factors, and the large molecular weight products produced by the cells remain in the ECS.

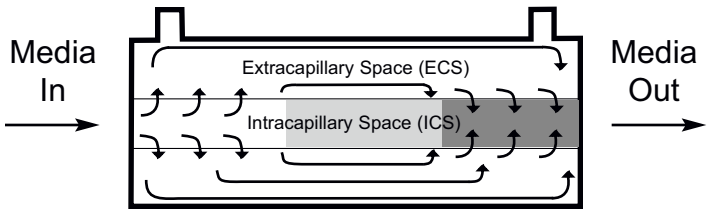


Figure 2-1. Hollow fiber in a bioreactor

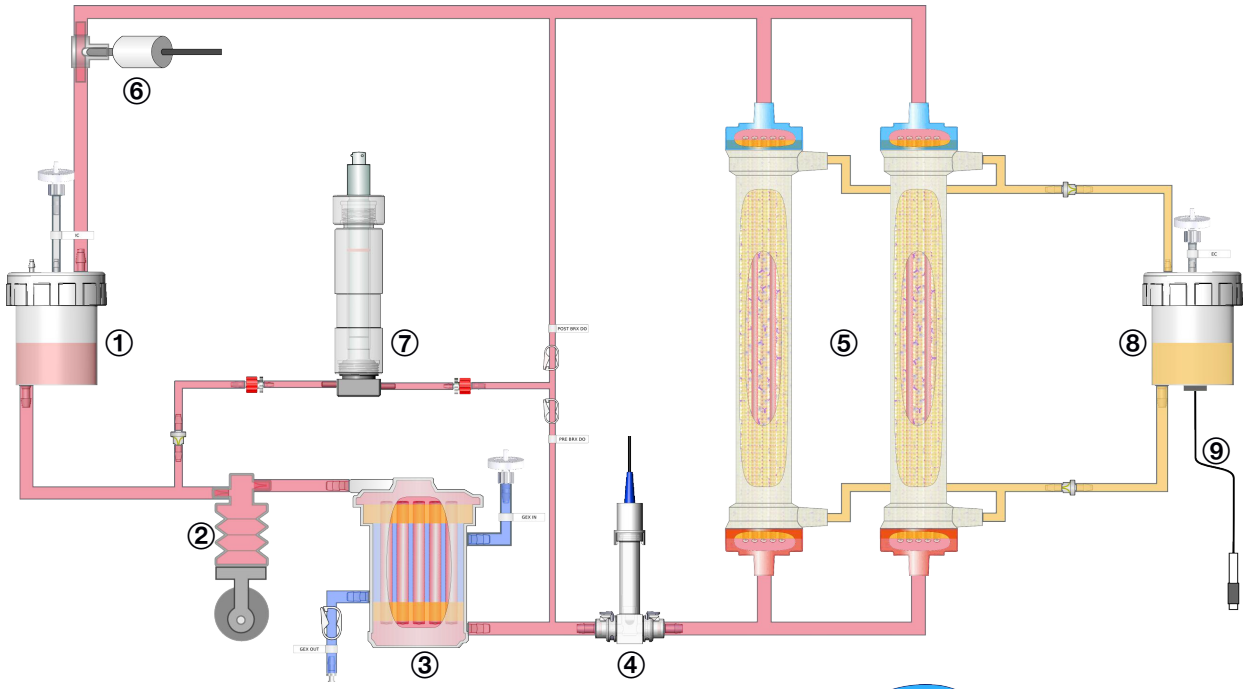
As the circulation pumps moves medium into the hollow fiber, a high relative pressure is created within the ICS. This high pressure forces medium through the pores of the fiber and into the ECS (Figure 2-1). As medium flows into the ECS, the pressure in the ICS drops along the length of the fiber. Near the distal end of the BRX, high relative pressure in the ECS forces medium to flow back into the ICS, carrying with it the metabolic waste from the ECS. This process is known as the Starling Effect and can be detrimental to the culture as it ends up pushing growth factors and cells to the distal end of the bioreactor.

Due to the ECS flow along the length of the BRX, cells at the entrance of the BRX are exposed to greater levels of oxygen and nutrients than those at the outlet end.

ACUSYST TECHNOLOGY DIFFERENCE

The *MAXIMIZER* compensates for Starling Effect and keeps the ECS flow channels open by a mixing process called EC Cycling. This mixing creates a more homogeneous cellular environment, allowing better cell growth and production.

Think of the *MAXIMIZER* as having two separate fluid circuits which intersect only at the fiber membrane of the BRX. These two fluid circuits, including their fluid reservoirs, constitute the **flowpath** of the system (Figure 2-2).



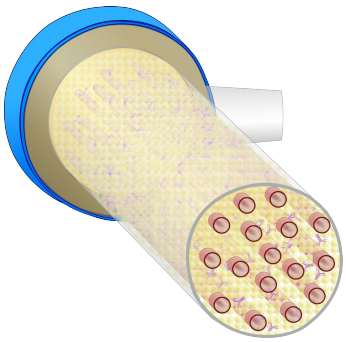


Figure 2-2. *Major Components of the Integration Circuit (red) and Expansion Circuit (yellow):*

① *Integration Chamber*
② *Circulation Pump*
③ *Gas Exchanger*
④ *pH Probe*
⑤ *Bioreactors (volume inside hollow fibers is IC, volume surrounding hollow fibers is EC)*
⑥ *Temperature Probe*
⑦ *Dissolved Oxygen Probe*
⑧ *Expansion Chamber*
⑨ *Volume Sensor*

The Integration Circuit

The Integration Circuit (IC) is the part of the flowpath which coincides with the inside of the fibers in the BRX. It includes:

- the circulation pump
- the Integration Chamber where media and factors are added to the system and waste is removed
- the GEX (oxygenator)
- the IC flowpath tubing
- the ICS of the BRX(s)
- the probes used for monitoring pH, DO and temperature of the system

Note: **Figure 2-2 has been simplified and does not show the DO probe because it is part of a secondary IC Circuit. See Figure 3-4 for a more detailed diagram.**

When the circulation pump is on, pressure is created in the IC which forces media to flow through the circuit and into the ECS from the ICS. This half of the flowpath is basically the same as in a conventional hollow fiber system. If left to circulate this way, the Starling Effect would create nutrient and waste gradients which would not permit efficient large scale production.

The Expansion Circuit

In the *MAXIMIZER* there is a second fluid circuit called the **Expansion Circuit (EC)**. This circuit is connected to the outside of the fibers where the cells are growing and product is being produced. The EC consists of:

- the **Expansion Chamber**
- two check valves which keep the flow within the circuit moving in one direction
- the ECS of the BRX
- the EC flowpath tubing

EC Cycling

In the first half of the cycle the IC is pressurized at the integration chamber. Additional pressure is provided to the IC by the circulation pump. As a result, medium flows from the ICS (inside of the fibers) to the ECS (outside of the fibers) along the length of the BRX, thereby moving media from the IC to the EC. Thus, cells are provided with fresh glucose, glutamine, dissolved oxygen and other small molecular weight nutrients. As media move from the IC to the EC, the media level in the integration chamber decreases and the media level in the Expansion Chamber increases. This half of the cycle is therefore referred to as "EC Rise".

When fluid in the Expansion Chamber reaches a defined upper limit, an ultrasonic detector signals the microprocessor to lower the pressure in the integration chamber. This reduces the pressure throughout the IC. In addition, pressure is applied to the Expansion Chamber to make the EC pressure higher than the IC pressure. As a result, medium flows from the EC to the IC, and removes small molecular weight wastes such as lactate and ammonia. This portion of the cycle is called "EC Fall". When the level of media in the Expansion Chamber reaches its defined minimum level, the process starts over.

Figures 2-3 and 2-4 illustrate this process. Arrows are shown to indicate net flow of media.

This alternating flow of media between IC and EC is called **EC Cycling**. This patented process thoroughly mixes the media in the BRX ECS and greatly reduces the microenvironments and gradients.

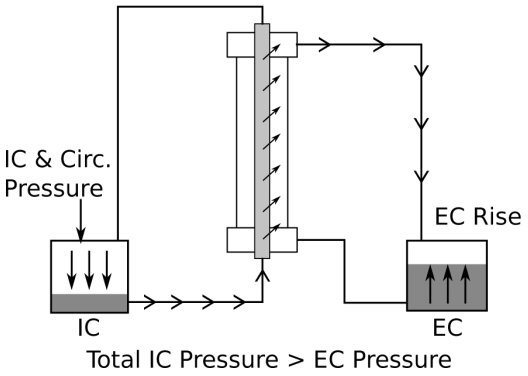


Figure 2-3:

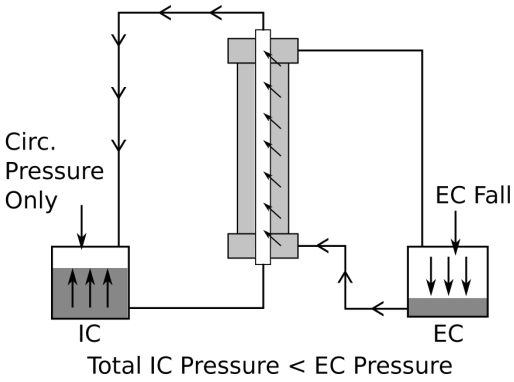


Figure 2-4:

Note: **Process controls in the ACUSYST-MAXIMIZER allow you to select time periods for the EC Rise and EC Fall phases of cycling as well as a desired transfer volume between IC and EC. To maintain these conditions, the instrument uses an ultrasonic level sensor to measure fluid level in the Expansion Chamber, and automatically regulates the pressures applied to the Integration and Expansion Chambers.**

This section will introduce you to the basic components of the ACUSYST-*MAXIMIZER*. Hardware includes the instrument and its obvious features, while cultureware includes the disposable flowpath and the reusable probes and holders. Also included in this section are descriptions of the bottles, cap assemblies, and optional printer package.

HARDWARE COMPONENTS

Refer to Figure 3-1 for front external view of *MAXIMIZER*.

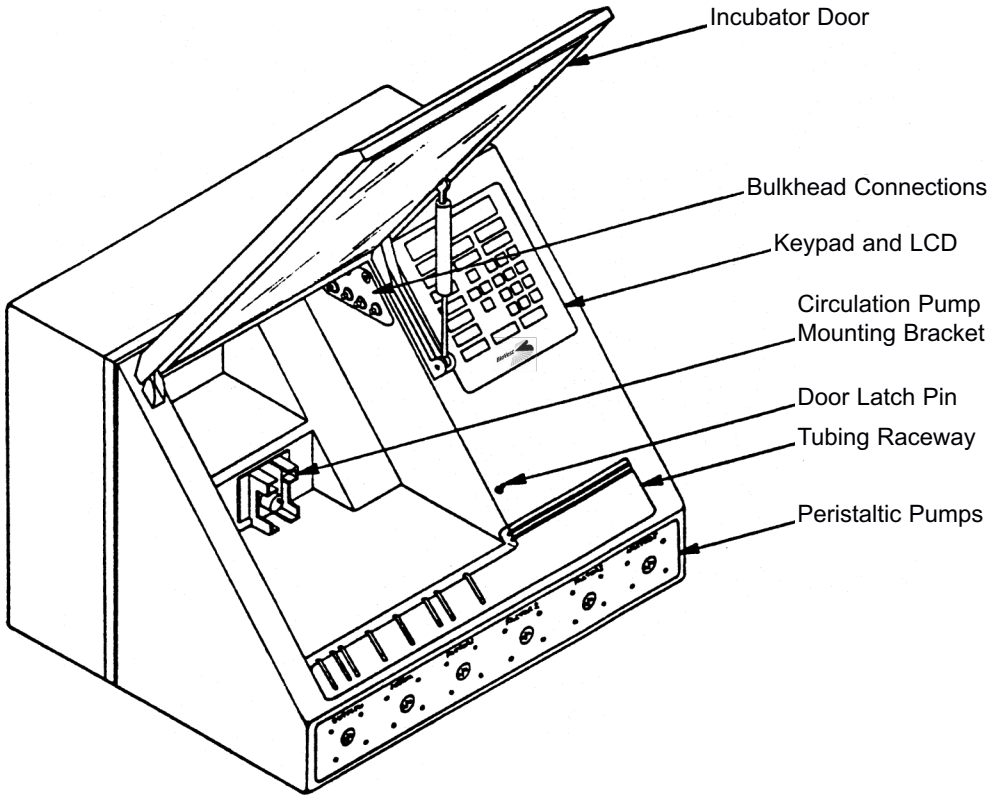


Figure 3-1. Front view of ACUSYST-MAXIMIZER (door open)

Instrument Cabinet

The cabinet of the *MAXIMIZER* is made of molded plastic. It may be cleaned with a mild detergent or alcohol cleaner. (For necessary counter space, see Section 4, Instrument Setup, for facility requirements.)

Incubator

The incubator module is a forced-draft convection system designed to maintain the culture at a controlled temperature. The exhaust fan for the incubator is located on the upper rear of the incubator. Access to the interior of the incubator is gained by lifting the door from its lower right hand corner. When the door is fully open, the incubator fan and heater are shut off. A viewing window on the door can be exposed to examine the incubator without disturbing its contents. Inside the incubator are two rows of **bulkhead** connections on the upper right wall (Figure 3-2). These are used in conjunction with **cultureware** to monitor and control various parameters of the culture.

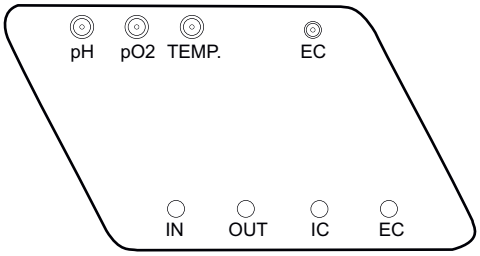


Figure 3-2. Bulkhead connections in the incubator.
Upper row connections are for electrical cables.
Lower row connections are for gassing lines.

Note: **To prevent over-heating, the temperature probe supplied with the MAXIMIZER should be connected to its bulkhead connection whenever the instrument is on and the incubator door is closed.**

In the center of the incubator floor is the mounting bracket for the circulation pump. Tubing can be routed in and out of the incubator by placing it in the **tubing raceway** panel below the door.

The *MAXIMIZER* is shipped with 4 pieces of Tygon tubing with color-coded Luer-Lok collars. They are designed to connect various parts of the flowpath module to bulkhead ports inside the incubator. These are **reusable** and should **not** be discarded.

Pump Modules

Six mounting brackets are located below the tubing raceway panel. These are labeled for their respective **peristaltic pump heads**: Outflow, Medium, Factor 1, Factor 2, Factor 3, Harvest. Two size 7016 and four size 7014 pump needs are included with the *MAXIMIZER*. Each pump head is packaged with a set of four mounting screws and a pump key. The Circulation pump is a plastic bellows assembly that is part of the flowpath module. It is mounted inside the incubator with two metal screws and a threaded plastic knob.

Keypad and LCD

To the right of the incubator is the control panel for the microprocessor. It consists of a touch sensitive keypad and a liquid crystal display (LCD). Separate buttons are used to activate the MONITOR screen, CALIBRATE function, ALARM listing, SETUP functions, PROCESS CONTROL parameters, and the INOCULATE procedure. In addition, a row of buttons is available to control certain OVERRIDE functions. The four arrow buttons are used to move the blinking cursor when it is accessible, and the numeric section of the keypad is used to ENTER or CLEAR values when accessible fields are shown in the LCD. The START and HOLD buttons at the lower left are used for special situations. Details on keypad operation are given in Section 5, **User Interface**.

Electrical and Gassing Features

The rear of the instrument contains several features necessary for the operation of the instrument (Figure 3-3). The power cord is attached to the lower left near the on/off rocker switch. Immediately to the right of the power switch is an access panel for the fuse. An alarm with volume adjustment ring is located in the lower center of the instrument. A 36-pin parallel port is available for attachment of the printer cable (optional). An air inlet and an exhaust fan, which cool the system electronics, are located in the lower right and upper left, respectively.

Mounting holes for the coalescent gassing filters are identified with a diagram on the lower right. One of these filters will be attached to the internal air compressor, while the other filter will be attached to an external supply of 100% carbon dioxide (CO2). Two fittings are provided for a backup air supply and an outgassing connection (both optional).

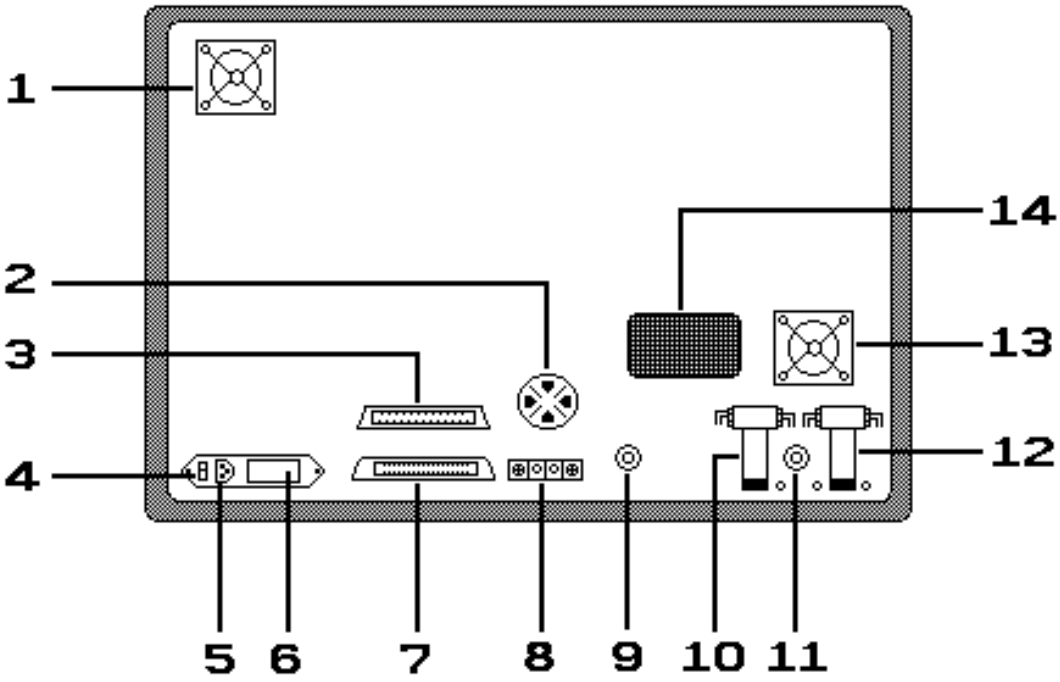


Figure 3-3. Rear view of ACUSYST-MAXIMIZER showing the main features: 1 = exhaust fan, 2 = alarm, 3 = serial interface port , 4 = on/off switch, 5 = power cord outlet, 6 = fuse, 7 = parallel printer port, 8 = alarm relay, 9 = offgassing port, 10 = air filter, 11 = backup air port, 12 = carbon dioxide filter, 13 = inlet fan, 14 = label for gas filter assembly.

CULTUREWARE COMPONENTS

The disposable part of the cultureware is the flowpath module, which consists of a plastic tray that holds the BRX, gas exchange cartridge, bellows-type circulation pump, two vessels called Integration Chamber and Expansion Chamber, plus associated tubing, **clamps** and **vent filters**. The module is self-contained and pre-sterilized.

The reusable parts of the cultureware are the oxygen and pH probe assemblies. They are to be steam sterilized then attached to the flowpath under a laminar flow hood.

Each flowpath module is supplied with an accessories package. This contains 60 ml syringes (for serum pre-treatment and inoculation), sterile sampling port septa, and tubing assemblies for the pH and DO probe cases.

Flowpath Modules

The flowpath consists of two major circuits that meet at the hollow fiber BRX (see Figure 3-4). The IC contains the Integration Chamber, circulation pump, pH probe assembly, oxygenator, medium tubing, outflow tubing, two **factor** lines, and the lumen of the BRX fibers. Within this major circuit lies a secondary path that contains the oxygen probe assembly and its associated tubing, plus one **sample port**. The EC contains the Expansion Chamber, harvest tubing, factor tubing, check valves, two access ports (for sampling or inoculation), and the BRX volume outside the hollow fibers (known as the ECS).

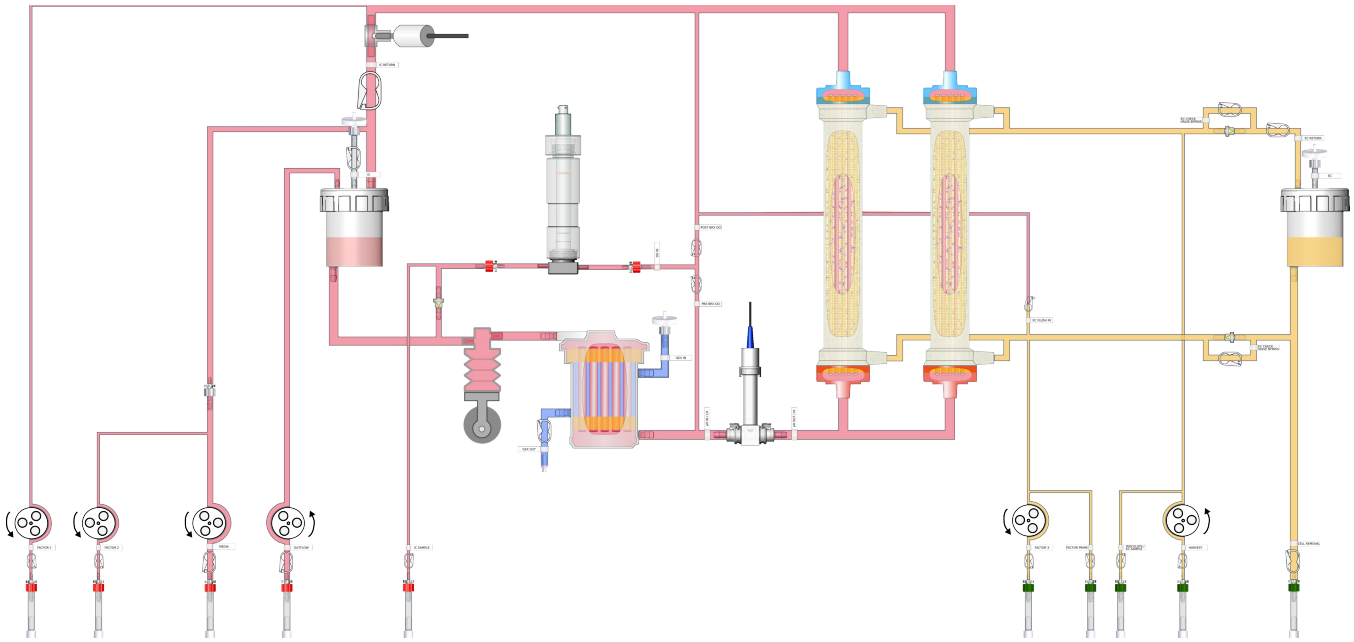


Figure 3-4. Schematic diagram of ACUSYST-MAXIMIZER flowpath
Intracapillary Circuit shown with red tubing
Extracapillary Circuit shown with yellow-ish tubing

Note: **Biovest offers four flowpath options: a single 1.5 m^2 or 2.1 m^2 bioreactor (not shown in the figure above) or double 1.5 m^2 or 2.1 m^2 bioreactors (shown in the figure above). See Appendix A for further specifications.**

The circulation pump moves media through the IC, while the cycling process causes media to flow through the EC and between the IC and EC. Depending on which IC sample clamp is open, IC medium flows through the DO probe from a site located upstream or downstream (PRE- or POST-) from the BRX.

Peristaltic pump tubing is made of PharMed, which is a highly durable material that will last throughout each run. The section of tubing that contains the DO probe and IC sampling port is made of medical grade Tygon to minimize errors in measuring DO.

An ultrasonic level sensor is attached under the Expansion Chamber to assist in the regulation of the cycling process by monitoring changes in fluid volume in the chamber. The drop tube in the Integration Chamber is connected to the Outflow pump, which runs continuously to remove excess media.

The silicone membrane oxygenator that serves as the primary pH control device. Regardless of the gas mixture used for pH control, the oxygenator provides near-saturation levels of dissolved oxygen to the IC. The oxygenator also contains a connector for the temperature probe.

Oxygen Probe and Case (Optional)

The *MAXIMIZER* can support a polarographic oxygen electrode. When it is inserted into its plastic and stainless steel DO probe case, it is considered a DO probe case **assembly** that is to be prepared for attachment to the flowpath. The case exposes the tip of the electrode to a small volume of constantly flowing medium for optimal response time. An electrical cable is provided to connect the probe to the appropriate bulkhead port in the incubator. When the electrode is not in use, it should be stored in a vertical position with the membrane tip at the bottom. The protective cap should cover the membrane tip, and the electrical cover should be in place.

Contact Account Services for more information.

pH Probe and Case

A polarimetric pH electrode is furnished with a container of buffer. When the electrode is inserted into its plastic and stainless steel pH probe case, it is considered a pH probe case **assembly** very much like the oxygen probe case assembly. The case is designed to expose the tip of the pH electrode to a small volume of constantly moving medium for rapid response time. When the electrode is not in use, it should be stored with the electrode tip in a buffer solution (pH = 4) and with the electrical cap in place.

BOTTLES AND CAP ASSEMBLIES (OPTIONAL)

Bottles

Biovest International offers glass bottles to be used with the *MAXIMIZER*. These bottles are provided with screw caps and plastic pouring rings that will survive repeated autoclave cycles (Figure 3-5).

Cap Assemblies

In addition to the bottles offered by Biovest International, two types of patented vented RESCU-CAP assemblies are available (Figure 3-5). Each type can be fitted with a 0.2 micron Acrodisc vent filter and two Luer plugs to ensure protection of sterility after steam sterilization. One type of assembly, the receiving cap, has a short segment of tubing attached to the inlet port. Bottles used for collection of outflow or harvest should have this type of cap assembly. The other type of assembly has a longer segment of tubing plus a glass tube on the outlet port. Bottles used to deliver (feed) medium to the flowpath should have this type of cap assembly with the correct length of tubing.

Figure 3-5. Bottle components and patented cap assemblies.

PRINTER PACKAGE (OPTIONAL)

A printer and communications cable may be purchased from Biovest International (see Appendix B). This device may be used with the *MAXIMIZER* to record process control events and alarms with a user-defined logging schedule. It is useful when hardcopies are needed for compliance with regulatory agencies, or for historical purposes (run-to-run comparison, troubleshooting, daily flowpath variance in process control, etc.). Included in this package is a box of printer paper compatible with the tractor feed mechanism.

SERIAL INTERFACE PORT

Optionally connect to this port either of the following two devices:

- ACULINK-DATA LOG™ Base Unit to electronically collect the run log and graph logged run data (see Appendix B for ordering information)

  or

- A serial-communications, tractor-fed printer (Biovest does not supply this type of printer)

This section will explain what is needed to install the ACUSYST-*MAXIMIZER* in the laboratory. It will describe the specific electrical and gassing requirements, as well as the procedures to test the instrument for damage that may have occurred during shipping. Please remember to save the packing list that was enclosed with the box of accessories.

FACILITY REQUIREMENTS

Bench space

The *MAXIMIZER* should be placed on a level surface in a room with ambient temperature of 18-26°C (64-79°F). If possible, the location of the *MAXIMIZER* should provide easy access to an autoclave and a laminar flow hood. The tabletop dimensions of the instrument are 69.8 cm [27.5 inches] (width) and 66 cm [26 inches] (depth). The height is 52 cm (20.5 inches) with the door closed, and 73.6 cm (29.0 inches) with the door open. To permit clearance of cables, gas lines, and the exhaust fan, the rear of the instrument must have clearance of 10 cm (4 inches). More space will be needed if the printer package is used. See Figure 4-1 for benchtop area covered by the *MAXIMIZER* and printer.

Electrical and Gassing Requirements

One electrical outlet is required for the *MAXIMIZER*. The *MAXIMIZER* may be configured at the factory for the three types of power specifications shown in Figure 4-2 (refer to Appendix A and B). The in-house electrical circuit must supply at least 500 watts of uninterrupted power. If the printer is used, an additional outlet will be needed. It is recommended that an uninterruptible power supply (on-line 500 vA with sine wave form) should be used with the *MAXIMIZER*.

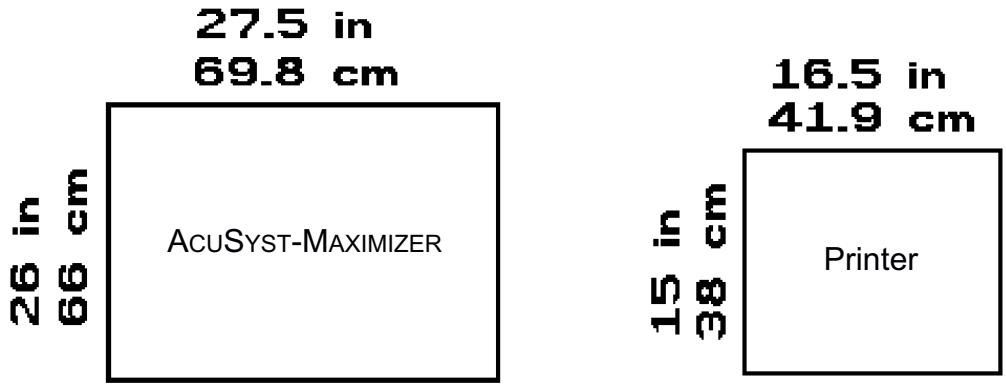


Figure 4-1. Foot print dimensions for ACUSYST-MAXIMIZER and printer

SPECIFICATIONS			
VOLTAGE	120 VAC	100 VAC	230 VAC
CURRENT	4 amp	4 amp	2 amp
FREQUENCY	60 Hz	50/60 Hz	50/60 Hz

Figure 4-2. Power requirements for the ACUSYST-MAXIMIZER

Attach the two gas filters to the rear of the instrument, as shown by the label (Figure 4-3, item 14). Remove the gas line plugs, and connect the gas line to the proper inlet and outlet. Cable ties should be used to secure all gas line connections. Attach the supply of 100% carbon dioxide (medical grade) to the correct filter, and set the incoming supply pressure to 15 psi (1.0 kg/sq.cm). A port is available for attaching a barbed fitting to be used for backup air supply (set to same pressure). If there is a need to filter or incinerate exhaust gas, an outgas port is available for a fitting and vacuum line.

Gas supply: Medical grade carbon dioxide (100%) is recommended for the *MAXIMIZER*.

Laboratory Supplies

See **Electrical and Gassing Requirements** for making the proper pressure adjustments and connections to the instrument.

Cell culture equipment: Various supplies will be needed to maintain the *MAXIMIZER* during a run. Laboratory coats, sterile gloves, masks, and other apparel are recommended to minimize the risk of contamination. The importance of these types of precautions depend upon the cleanliness and sterility of the room where the *MAXIMIZER* is located, as well as the presence or lack of antibiotics in the culture medium. Inoculation will require sterile syringes (60 ml) and sterile needles (15 gauge). Two sizes of sterile syringes (10 ml and 3 ml) are recommended for sampling; if septa are used during sampling, sterile needles (20 gauge) will also be needed. A disinfectant solution of alcohol, beta-iodine, peracetic acid, etc. is necessary for making sterile connections. Sterile gauze or cotton pads may be used with the disinfectant to protect the culture during these procedures.

A laminar flow hood, autoclave, and some kind of pH meter (benchtop monitor, or blood gas analyzer) are absolutely required for certain procedures. Assay equipment and reagents for the measurement of secreted product, glucose and lactate are used to adjust process control parameters. Calibration of the optional oxygen electrode can be performed offline with an instrument such as a blood gas analyzer.

PRINTER SETUP (OPTIONAL)

Biovest International optionally offers a printer to document the run log. It is used with the *MAXIMIZER* to make hardcopy records of process control conditions and alarms during the run. Assemble the printer and tractor feed according to the printer's pre-packaged instructions, and connect the printer to the *MAXIMIZER* by attaching the parallel interface cable (see Figure 4-3 for location of parallel port). Load the printer with paper, and plug it into an appropriate electrical outlet (or uninterruptible power supply, as described on page 4-1).

SERIAL INTERFACE PORT

The serial interface port is a 25-pin serial cable connector on the rear panel of the instrument. The serial port provides for the transfer of information from the *MAXIMIZER* to an IBM-compatible computer or a serial printer. The *MAXIMIZER* sends the same information to the parallel and serial ports.

An advantage of using a serial printer, rather than a parallel printer, is that a serial printer may be placed an extended distance from the instrument for remote printing - outside the cleanroom environment, for instance. One advantage of using a computer over a printer is that, unlike printouts, a computer can capture the printout as data. This allows the run data to be collected without using particulate-generating paper.

Other advantages of using a computer are selective reviewal, graphical analysis, and archiving of run data, and remote monitoring of instrument operation. Biovest International offers ACULINK-DATA LOG™, a custom hardware and software solution that runs on a PC computer to log run data and provide the benefits listed above. For computer information about the ACULINK-DATA LOG system, contact Biovest International's Account Services Department.

There are four modes available for parallel and serial port operation. These different modes allow the *MAXIMIZER* to adapt to the requirements and equipment of the production facility. The differences between the four modes are described below. Refer to Figure 4-3 and 4-4 for details of configuring the desired mode in the *MAXIMIZER* instrument. The factory default configuration of the *MAXIMIZER* is parallel printer mode at a data transmission speed of 9600 baud.

Caution: **Contact Biovest International's Account Services Department if another mode of operation is necessary. You risk instrument damage and/or personal injury by changing the rocker switch settings!**

	Rocker Switch Settings	
Desired Mode	Switch 3	Switch 4
Parallel printer only	Open	Open
Serial printer only	Closed	Open
Serial computer	Open	Closed
Restricted mode	Closed	Closed

Figure 4-3. Serial interface board settings for printer and computer

Rocker Switch Settings		
Switch 1	Switch 2	Baud Rate
Open	Open	9600
Closed	Open	4800
Open	Closed	2400
Closed	Closed	1200

Figure 4-4. Serial interface board settings for baud rate.

To use a serial printer configure its communications for 8 data bits, no parity, and 1 stop bit and to use XON/XOFF flow control. The serial port uses only transmit, receive, and ground signals (Figure 4-5). The printer must be compatible with the printer's control characters.

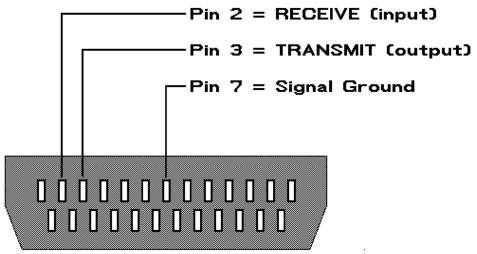


Figure 4-5. Location of main pin functions on serial interface port

Parallel Port and Serial Port Modes of Operation

Parallel printer mode is primarily meant for only parallel printing, but the serial port is active for use by the optional ACULINK-DATA LOG system (see serial computer mode).

Serial printer mode allows simultaneous printing from both parallel and serial printers. A parallel printer must be used in serial printer mode. The serial printer may be placed at a remote location - outside the production room, for instance, for convenience or other purposes.

Serial computer mode is primarily meant for only using the optional ACULINK-DATA LOG system. The serial computer mode does, however, allow the use of a parallel printer. The serial port will override any parallel printer failures after a short timeout.

Restricted mode allows simultaneous printing from both parallel and serial printers. A parallel printer may be used in serial printer mode. The serial port will override any parallel printer failures after a short timeout.

AcuLink-Data Log™ SETUP (OPTIONAL)

Biovest International optionally offers AcuLink-Data Log, a custom hardware and software solution that runs on a PC computer to electronically collect and store run data, rather than printing this information, as described above.

Advantages of using AcuLink-Data Log include:

- saving space in the clean room
- eliminating particulate-generating paper in the clean room
- faster, simpler review of log data
- archiving run log data
- faster, simpler monitoring or instrument operation
- improving reliable documentation of log data

Contact Biovest International's Account Services Department for further information about AcuLink-Data Log.

This section describes the control features and screens that are accessed using the keypad of the ACUSYST-*MAXIMIZER*. Some of the keys and features are not explained in detail in this chapter, but a brief description of their general use is provided. More extensive coverage is provided in the chapter 6, **Operating Procedures**. This is necessary for some features because it is easier to learn them as you proceed through the steps of a culture run. Guidance for using some of these features is provided in chapter 6, **Optimization Procedure.**

Note: **Refer to Section 7, Troubleshooting for a complete list and description of alarms.**

OVERVIEW OF LCD AND KEYPAD

Refer to Figure 5-1 for the location of the LCD and keypad items.

LCD

This is the screen at the top of the keypad. Information is displayed as discrete screens that may or may not contain a flashing, movable cursor. Because the LCD has space for a limited amount of text, some information must be presented as a series of separate screens. Scroll through them by repetitively pressing **ENTER** (for most series) or ⬅ and ➡ (for others).

Keypad

Numeric Panel: This is a collection of keys numbered **0** through **9**, with a key for decimal (**.**), **CLEAR**, and **ENTER**. A selection from a menu is chosen by pressing the desired number. To input values, such as pH or temperature set points or various pump speeds, first press the desired number keys, then press **ENTER**. If an error is made before **ENTER** is pressed, press **CLEAR** to erase the unwanted entry.

Menu Keys: The following keys are called menu keys because pressing them displays a list (menu) of items to be selected for further action:

- **SET-UP**
- **PROCESS CONTROL**
- **CALIBRATE**
- **HOLD**
- Four Override Control keys

LEDs are present in the upper, left-hand corner of some keys. They are red when turned on. Their purposes are explained with their relevant keys in the sections throughout this chapter.

Arrow keys move the cursor to fields that need input, or to the override indicator (*) if it is present. The cursor uses the "wrap-around" function as a shortcut function to quickly move from one end of the screen to another end, if desired (for example, pressing ⬆ from a topmost field brings the cursor to a bottommost field).

The ⬅ and ➡ keys are also used with **ALARM** screens to scroll through the stored alarm messages.

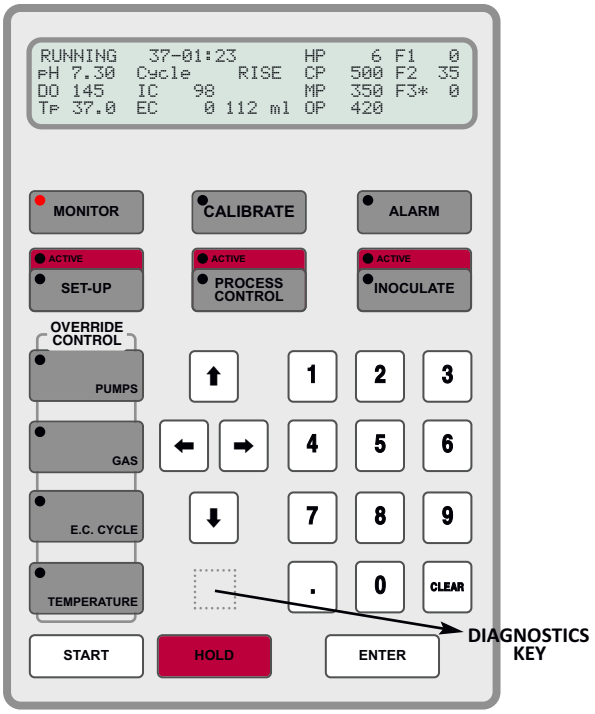


Figure 5-1. ACUSYST-MAXIMIZER keypad and LCD

START has several functions. **START** must be pressed immediately before pressing **SET-UP**, **PROCESS CONTROL**, or **INOCULATE** to activate these functions and cause the respective LED to turn on to confirm which of the three functions is active. Only one of these functions may be active at any one time (see caution note below).

To display the series of diagnostics screens, press **START** then the Diagnostics key (an unlabeled key). Diagnostics screens may be displayed at any time during the run, so the caution note below does not apply to the diagnostics screens.

Lastly, **START** also is used during the calibration procedure according to the onscreen instructions.

CAUTION: Be careful about pressing START! See caution note on page 5-7. If START is accidentally pressed, press MONITOR or CLEAR before pressing another key.

MENU KEY OPERATIONS

MONITOR Key

Pressing **MONITOR** displays the main screen that shows most of the parameters that are automatically monitored and/or controlled (see Figure 5-2).

Figure 5-2. MONITOR screen

The Monitor screen shows which of the four modes of operation (IDLE, SETUP, INOC, and RUNNING) is currently active and for how long the mode of operation has been active, as shown by the elapsed time. Elapse time is represented as days-hours:minutes with maximum values of 999 days, 23 hours and 59 minutes. Elapsed time resets to 0-00:00 whenever a new mode of operation is actived. Elapsed time represents relative elapsed culture time, not the time of day or date.

The remaining information on the Monitor screen is shown for all four modes of operation and represents currently monitored/controlled conditions:

PH = pH of pre-BRX medium
DO* = dissolved oxygen (mmHg)
Tp = temperature (°C) of circulating medium
CYCLE = phase of EC Cycling currently active (RISE, FALL, LOW, or HOLD)
IC = pressure (mmHg) in Integration Chamber
EC = pressure (mmHg) and volume (ml) in Expansion Chamber

HP = Harvest pump rate (ml/hr)
CP = Circulation pump rate (ml/min)
MP = Media pump rate (ml/hr)
OP = Outflow pump rate (ml/hr)
F1 = Factor 1 pump rate (ml/hr)
F2 = Factor 2 pump rate (ml/hr)
F3 = Factor 3 pump rate (ml/hr)

Note: *** The DO value displayed on the Monitor (and Calibrate) screen may change depending on whether the pre-BRX or post-BRX clamp is open (see Figure 3-4). The DO value on the LCD does not reflect which of these clamps is open, nor is this indicated in the run log.**

Following is a synopsis of the four modes of operation and the sequence of when they normally occur during a culture run.

IDLE appears on the Monitor screen immediately after the Abort function is used (when the previous run was terminated) and until one of the active function begins: either **ACTIVE SET-UP**, **ACTIVE PROCESS CONTROL**, or **ACTIVE INOCULATION**.

SETUP appears on the LCD after pressing **START** then **SET-UP**, which also turns on the Active Set-Up LED and resets elapsed time to 0–00:00. Start Set-Up activities normally occur after Idle. Further information about Start Set-Up is described three pages below.

RUNNING appears automatically after completing the Start Set-Up activities, and the Active Process Control LED also turns on automatically. Elapsed time resets to 0–00:00. This stage of the run is called Pre-Inoculation phase, a time when the *MAXIMIZER* is adjusting the cell culture medium to make it ready for inoculating the cultureware with the scale-up culture by controlling pH, temperature, EC Cycling, etc. Alarms, printing, and other functions become enabled when the *MAXIMIZER* is in RUNNING mode.

INOC appears after pressing **START** then **INOCULATE**, which also turns on the Active Inoculate LED. Elapsed time resets to 0–00:00. Inoculation phase is a short-duration phase used to inoculate the cultureware with the scale-up culture.

RUNNING re-appears automatically after completing the Start Inoculate activities. The Active Process Control LED turns on automatically again. The *MAXIMIZER* resumes controlling process control parameters programmed in the series of Process Control screens.

MAXIMIZER remains in RUNNING mode until it is time to terminate the run. Press **HOLD** then **2** to abort the run. IDLE now appears on the screen, and the cycle of progressing through these modes of operation repeats for the next culture run.

Note: IDLE, SETUP **and** INOCULATE **must be manually activated.** RUNNING **normally appears (and becomes the active mode) automatically after Fill/Flush and Inoculation procedures are completed. However, if you need to directly activate** RUNNING **mode, press *START* then *PROCESS CONTROL*. The Active Process Control LED turns on; elapsed time resets to** 0-00:00**; and pH, temperature, EC Cycling and other process control functions become active.**

Override Controls are available during any of the four modes of operation described above. If an override applies to a parameter displayed on the Monitor screen, its status of being manually overriden is indicated by an asterisk (*) to the left of the monitored parameter. An example of this is shown in figure 5-2. The F3 pump is overriden to a speed of 0 ml/hr.

SETUP Key

Press **SET-UP** to access two screens: `System Tests` and `Logging Interval` (figures 5-3 and 5-4). Whichever screen was last viewed is the first one to be displayed. Press **ENTER** to cycle between the two screens. Press **MONITOR** to exit from either screen. These screens may be accessed at any time during an active culture run.

System Tests: Six instrument self-tests should be performed on the *MAXIMIZER* before each culture run is started. These tests ensure the functioning of the LCD, audible alarm, LEDs, keypad response, and printer connection. The sixth test allows you to examine the revision level of the microprocessor PROMs.

```
System Tests:
1) LCD       2) Alarm     3) LEDS
4) Keypad    5) Printer   6) PROMS
```

Figure 5-3. System Tests Menu Screen

Instrument Setup Procedures: (beginning on page 6-15) explain the details of the six System Tests, which are activated by pressing the respective menu numbers.

Printer Log Interval: The second Set-Up screen is shown in the figure below.

```
Printer: Disabled

Logging Interval: _ 0 mins (0-255)
```

Figure 5-4. Logging Interval Screen

Logging Interval is the user-defined frequency for sending current instrument status information to the printer or ACULINK-DATA LOG. When set to `0`, logging is disabled. The interval may be changed at any time during an active culture run. Logging is active only in `RUNNING` mode. This feature applies to a printer or ACULINK-DATA LOG connected to the serial or parallel port.

Note: **The function of Logging Interval is different from the Printer test in the System Tests screen.**

START/SETUP Functions

Press **START** then **SET-UP** to display the screen shown in figure 5-5. This also causes the Active Set-Up LED to turn on, and it changes the miniMAX's status from IDLE to SETUP. Press **MONITOR** to exit from this screen.

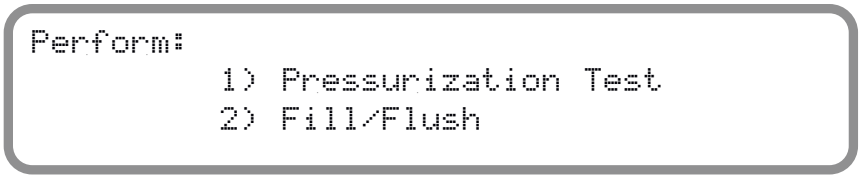


Figure 5-5. START/SETUP Selection Screen

Pressing **1** or **2** results in several screens of instructions that are specific to the chosen procedure. The PRESSURIZATION TEST must be performed before FILL/FLUSH! After the Pressurization Test is complete, the display returns to this screen in order to then perform Fill/Flush. See chapter 6 for operational details.

Pressurization Test: This program applies air pressure at the EC and IC bulkhead ports (and anything connected to them, such as pH or DO probe assemblies or the entire cultureware), then measures any detectable reduction as a sign of leakage. The test is performed several times per culture run to confirm the *MAXIMIZER* instrument, probe assemblies, and cultureware are leak-free.

Fill/Flush Test: This program is used to fill the cultureware and rinse the wetting agent from the hollow fibers in the bioreactor cartridge in preparation for the pre-inoculation phase.

CAUTION: During an active culture run the Monitor screen shows RUNNING as the run mode and the LED for Active Process Control is turned on. During an active run, do NOT press START then SET-UP because this will stop the active run and display the screen shown in figure 5.5! However, if this is accidentally done, press START then PROCESS CONTROL to restart the active run. Process Control settings will be retained (if the backup battery is still good), but elapsed time will reset to 0-00:00, which would affect process control parameters that include a delay time feature. Press PROCESS CONTROL and review all screens to ensure their settings return the instrument's operation to what it was prior to accidentally deactivating the run.

PROCESS CONTROL Key

Press **PROCESS CONTROL** to access several screens to enter or review the settings that define how the *MAXIMIZER* operates when in RUNNING mode. The various screens include information on pH control, pump rates and pump schedules, alarm limits, temperature setpoint, and EC Cycling parameters. These parameters have specific default values, but they may be changed at any time during a culture run.

Pressing **PROCESS CONTROL** displays whichever of the series of the Process Control screens was last viewed. This also causes the Process Control LED (in the gray area of the key) to turn on. Repetitively press **ENTER** to scroll through the series of Process Control screens. To exit from any of these screens, press **MONITOR**.

Chapter 6 provides a detailed explanation of pH control, oxygenation, and basic strategy implemented in a culture run.

pH Setpoint and Gas Numbers: The screen below shows the current pH Setpoint that process control will attempt to maintain. This setpoint ideally is specific to the mid-log phase of growth that is specific to the cell line being actively cultured. This value may be changed anytime during the active culture run. Acceptable values are in the range of 6.00 – 8.00 pH units, in increments of 0.01 units.

```
pH Setpoint:      7.10

                 LOW    HIGH
GAS #:             1      14
```

Figure 5-6. pH and Gas Number Screen

This screen also allows you to limit the LOW and HIGH gas numbers. We strongly suggest using the default values: LOW 1, HIGH 14. A typical run needs the full range of gas numbers. When changed, the LOW value must be ≤ the HIGH value. (When LOW value = the HIGH value High, the result is the same as overriding Gas # to a single gas number.)

Note: **Changing the default LOW and HIGH values reduces the range of pH control.**

Alarms:

Use this screen to set the desired LOW and HIGH alarm limits for pH, DO, and Temperature and setpoint for Temperature. These alarms are active only in RUNNING mode. To enter a temperature setpoint outside of the default alarm range, first change the LOW or HIGH temperature limit to a value outside of the setpoint.

Acceptable ranges and increments:

- pH alarm: 6.00 – 8.00 pH units 0.01 units
- DO alarm: 0 – 200 mmHg 1 mmHg
- Temperature alarm: 24.1 – 45.8°C 0.1°C
- Temperature setpoint: 24.1 – 45.8°C 0.1°C

```
      Set        LOW*ALARM*HIGH
pH               7.10        7.50
DO                 80          20
Tp   37.0        36.5        37.5
```

Figure 5-7. Alarm and Temperature Setpoint Screen

Circulation Rate:
The `Circulation Rate` screen allows you to set the speed of the Circulation Pump in ml/min. As described in chapter 6, **Oxygen Delivery**, this value should be raised during the active culture run to maintain a minimum desired post-BRX DO level (suggest 100 mmHg). Acceptable Circulation Rate values are between 0 – 1000 ml/min, in increments of 1 ml/min.

Figure 5-8. Circulation Rate Screen

Note: **Cultureware that contains one BRX should not exceed circulation rates of 600 ml/min.**

Media Pump Rates:
The `Media Rates` screen defines two pump rates related to pumping IC medium by the Media Pump: `Basal` for nutrient feed, `Dilution` for optional, supplemental control of pH (see **pH Control** beginning on page 6-1). Acceptable rates (for both) are between 0 – 800 ml/hr, in increments of 1 ml/hr.

The Basal rate should be changed according to metabolic information — periodic off-line assays of media samples to measure, for example, glucose concentration. As the culture expands and requires faster nutrient delivery, increase the Basal rate, as necessary.

When the `Dilution` rate ≤ `Basal` rate, the Media Pump remains at the Basal rate to maintain the chosen nutrient delivery rate. The result is the disabling of the Medium Dilution method of maintaining the pH setpoint. Excess medium will not be used to dilute lactate even if pH drops below its setpoint.

When the `Dilution` rate > `Basal` rate, the result is the enabling of the Medium Dilution method of maintaining the pH setpoint. Excess medium may be used to dilute lactate if pH drops below its setpoint.

Figure 5-9. Medium Pump Rates Screen

Factor 1 (F1, Base Addition) Pump Rate:

This screen allows you to enable (press **1**) or disable (press **2**) the base addition strategy for pH control, and to designate a volume that must be pumped into the cultureware before the next strategy (Medium Dilution) is activated.

Base solution is automatically pumped at ½₀ of the Medium Pump rate, or 5 ml/hr, whichever is greater — when Gas #1 (typically) by itself is unable to maintain (or raise pH to) the pH setpoint. If Base Addition is enabled (option 1), you may enter 0 – 9999 ml (in increments of 1 ml) as the desired `Base Volume before start of Dilution`. This volume of base is pumped before the instrument activates Medium Dilution, if it is enabled and its use is necessary — pH continues to be lower than the setpoint despite base addition being used.

Base addition continues once Medium Dilution begins. If Base Addition is enabled and the `Base Volume before start of Dilution` is set to `0 ml` (as shown in Figure 5-10), then Base Addition and Medium Dilution (if enabled) begin simultaneously — when Gas #1 (typically) by itself is unable to maintain (or raise pH to) the pH setpoint.

After pH returns to the desired setpoint, Base Addition stops (F1 pumps stops turning) and the `Base Volume before start of Dilution` is reset to the user-defined volume, for example, 50 ml. If pH goes out of range again and Base Addition is activated, another 50 ml of base may be pumped (if pH remains below the setpoint during this time) before Medium Dilution can be activated.

```
Base (F1): DISABLED
1 = Enabled,  2 = Disabled   _2
Base Volume before
start of Dilution             0 ml
```

Figure 5-10. Factor 1 (Base Addition) Screen

If you do not need to pump base to assist with pH control (which is true for most applications) and instead want to use the F1 Pump to deliver some other solution into the IC, leave Base Addition disabled and control this pump's speed using a manual override. Press **PUMPS**, use the arrow keys to navigate to the F1 pump field, enter the desired pump rate using the number keys, and press **ENTER**.

EC Cycling:

This screen allows you to set the conditions used by the *MAXIMIZER* in regulating the fluid cycling process between IC and EC. Default values are:

- `Delay  07-00:00` (7 days)
- `Transfer Vol  70` (ml)
- `UP 15` (15-minute Rise time)
- `DN 15` (15-minute Fall time)

During active process control (`RUNNING` mode), the *MAXIMIZER* controls the volume in the Expansion Chamber at a low level setpoint (108 ml) during the EC Cycling Delay period. This is displayed as `LOW` on the Monitor screen. When the elapsed time exceeds the delay period, EC Cycling begins (in a Rise phase) using the transfer volume and Rise/Fall times specified in this process control screen. When the ultrasonic level detector senses that the desired transfer volume has been added to the Expansion Chamber, the Rise phase is stopped, and the Fall phase begins. At the low level setpoint, the Fall phase stops, and the sequence repeats.

Use ⬅⬆➡⬇ to move the cursor between the four programming options.

Allowed EC Cycling parameters (see chapter 6, Optimization Procedures):

- Delay: 0-00:00 – 999-23:59, in increments of 1 minute
- Rise Time: 5 – 999 minutes, in increments of 1 minute
- Fall Time: 10 – 999 minutes, in increments of 1 minute
- Transfer Volume: 1 – 140 ml, in increments of 1 ml

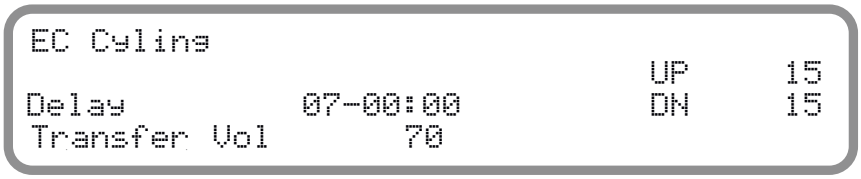


Figure 5-11. EC Cycling Parameters Screen

Factor 3 (F3, Serum) Pump Control: This pump delivers factor 3 solution (growth-supplemented medium) to the EC in a continuous or discontinuous (pulsatile) manner.

After the user-defined Delay period elapses, the pump runs at the desired flow Rate. The On and Cycle fields are used to define a continuous or pulsatile pumping process.

To run the F3 Pump continuously, the On and Cycle times must be set to the same, non-zero time. This is the most common mode of operation for this pump.

To run the F3 pump in pulses at your desired intervals, set the On field to the length of time the pump should turn, and set the Cycle field to the combined On and Off time. Pulses are the only means of achieving fractional rates, and they allow frequent or infrequent and large or small doses of factor to be pumped.

Example: Rate = 3, Delay = 2-12:30, Cycle = 00-02:00, On = 00-00:30. With these settings the F3 Pump will begin turning two days, twelve hours and thirty minutes after RUNNING mode began and at a rate of 3 ml/hr. The F3 Pump will turn for 30 minutes, stop for 1 hour 30 minutes (to complete the two-hour CYCLE time), turn again for another 30 minutes, stop again for another 1 hour 30 minutes, etc. The long-term, effective pumping rate of these pulses will be 0.75 ml/hr (3 ml/hr at 25% on-time).

Allowed F3 Pump parameters:

- Delay: 0-00:00 – 999-23:59, in increments of 1 minute
- Rate: 0 – 200 ml/hr, in increments of 1 ml/hr (see page A-4)
- On: 0-00:00 – 999-23:59, in increments of 1 minute
  On time must be ≤ Cycle time
- Cycle: 0-00:00 – 999-23:59, in increments of 1 minute

Figure 5-12. Factor 3 (Serum) Control Screen

Note: **The use of Cycle time in this screen should not be confused with — and is unrelated to — EC Cycling parameters described on page 5-12.**

Factor 2 Pump Control (F2): This pump delivers factor 2 solution (usually a glucose or glutamine concentrate) to the IC in a continuous or discontinuous (pulsatile) manner.

The usage for these settings is the same as for the F3 Pump Control, described on the previous page.

Allowed F2 Pump parameters:

- Delay: 0-00:00 – 999-23:59, in increments of 1 minute
- Rate: 0 – 200 ml/hr, in increments of 1 ml (see page A-4)
- On: 0-00:00 – 999-23:59, in increments of 1 minute
  On time must be ≤ Cycle time
- Cycle: 0-00:00 – 999-23:59, in increments of 1 minute

Figure 5-13. Factor 2 Control Screen

Note: **The use of `Cycle` time in this screen should not be confused with — and is unrelated to — EC Cycling parameters described on page 5-12.**

Harvest Schedule: This screen allows you to select the type of harvesting strategy to be employed by Process Control. One of the choices (option 4) is no harvesting. The other choices are referenced as Continuous, Continuous Batch, and Timed Batch and are described in detail on the following pages.

When any of the 4 selections are made, the top of this screen displays the name of the selection after `Harvest Schedule:`

When option 4 is chosen, the Harvest Pump does not turn and the next Process Control screen (displayed by pressing **ENTER**) is pH Setpoint.

When options 1 – 3 are chosen, the next Process Control screen (displayed by pressing **ENTER**) varies based on the harvest schedule option chosen, and it provides the appropriate settings needed for the chosen harvesting method.

```
Harvest Schedule: Continuous

1) Continuous         3) Timed Batch
2) Continuous Batch   4) None
```

Figure 5-14. Harvest Schedule Selection Screen

Note: **Because the Continuous Batch and Timed Batch strategies rely partly on EC Cycling to start the harvest pump, certain conditions related to EC Cycling may influence timing aspects of these two harvesting methods.**

- When the Delay time of EC Cycling is greater than the Delay time of the Continuous Batch or Timed Batch harvest strategy, these two harvesting methods will not begin until after EC Cycling has started.
- Using the Override Controls (described below) to set a Low or Hold condition on EC Cycling may prevent these harvest strategies from being used. This potential pause to harvesting depends on the phase of EC Cycling that is in progress when either of these two EC Cycling overrides is set.

Continuous Harvesting: When this selection is made, the screen in figure 5-15 will be displayed by pressing **ENTER**.

As with the F3 Pump and F2 Pump screens, you may select a `Delay` time and harvest pump `Rate`, as well as a continuous or pulsatile harvest pump operation.

Yes, even though this harvest method is called *Continuous*, it nevertheless is possible to program a pulsatile harvest pumping method (in addition to the fully continuous method). The basic difference between Pulsatile Continuous Harvesting and Continuous Batch or Timed Batch is Pulsatile Continuous Harvesting occurs irrespective of the EC Cycling process. In other words, the Harvest Pump operates regardless of the fluctuating volume in the Expansion Chamber. Continuous Batch and Timed Batch operate the Harvest Pump relative to the Expansion Chamber volume. See the following page for further details about Continuous Batch and Timed Batch.

Fully continuous harvesting is the most common method of operating the Harvest Pump. To achieve this, the `On` and `Cycle` times must be set to the same, non-zero time. If the `On` and `Cycle` times remain at the default of `0-00:00`, the Harvest Pump will not turn on, even when the `Delay` time is elapsed and the `Rate` is `>0 ml/hr!`

Allowed parameters:

- Delay: 0-00:00 – 999-23:59, in increments of 1 minute
- Rate: 0 – 200 ml/hr, in increments of 1 ml (see page A-4)
- On: 0-00:00 – 999-23:59, in increments of 1 minute
  On time must be ≤ Cycle time
- Cycle: 0-00:00 – 999-23:59, in increments of 1 minute

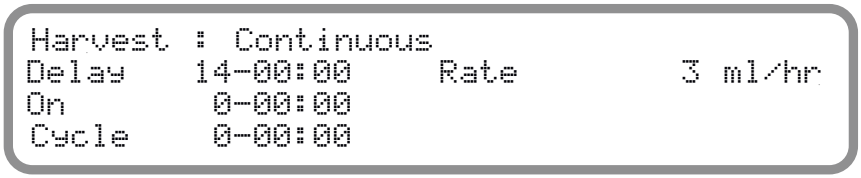


Figure 5-15. Continuous Harvest Control Screen

Note: **The use of Cycle time in this screen should not be confused with — and is unrelated to — EC Cycling parameters described on page 5-12.**

Continuous Batch Harvesting: When this selection is made, the screen in figure 5-16 will be displayed by pressing **ENTER**.

Once the user-defined `Delay` time has elapsed (and the separate EC Cycling Delay has elapsed), this harvesting method becomes active. The Harvest Pump then will run at a pre-programmed, high flow rate to remove the user-defined `Volume` **each time the Expansion Chamber volume is near its lowest level** during the EC Cycling's Rise and Fall process. Product concentration is highest when the Expansion Chamber volume is at its lowest level, as compared to when this volume is at its highest level.

Continuous Batch harvesting generally occurs more frequently than the Timed Batch method (see next page), although both harvesting methods remove fluid when the Expansion Chamber volume is near its low level.

Allowed parameters:

- Delay: 0-00:00 – 999-23:59, in increments of 1 minute
- Volume: 0 – 35 ml, in increments of 1 ml

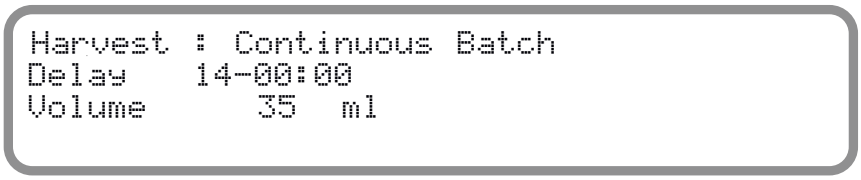


Figure 5-16. Continuous Batch Harvest Control Screen

Timed Batch Harvesting: When this selection is made, the screen in figure 5-17 will be displayed by pressing **ENTER**.

Once the user-defined `Delay` time has elapsed (and the separate EC Cycling Delay has elapsed), this harvesting method becomes active. The Harvest Pump then will automatically run to remove the user-defined `Volume` each time the Harvest Pump turns on, which generally **does NOT occur each time the Expansion Chamber volume is near its lowest level** during the EC Cycling's Rise and Fall process.

Unlike with Continuous Batch harvesting, Timed Batch harvesting includes a `Cycle` time. This `Cycle` time repeats, counting down to `0-00:00` repeatedly. Once `Cycle` time reaches `0-00:00`, the Harvest Pump waits until the next time the Expansion Chamber volume is near its low level. Then the Harvest Pump turns on at the pre-programmed, high flow rate to remove the user-defined Volume.

Several EC Cycling Low Levels may occur without the Harvest Pump turning on while the Harvest Pump is waiting for the Timed Batch Cycle time to count down to `0-00:00`. In this way, Timed Batch harvesting generally occurs less frequently than Continuous Batch harvesting, although both batch methods remove fluid when the Expansion Chamber volume is near its low level.

Allowed parameters:

- Delay: 0-00:00 – 999-23:59, in increments of 1 minute
- Volume: 0 – 35 ml, in increments of 1 ml
- Cycle: 0-00:00 – 999-23:59, in increments of 1 minute

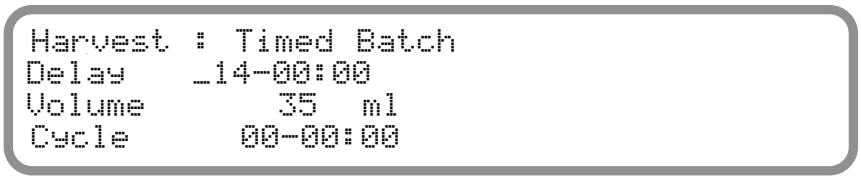


Figure 5-17. Timed Batch Harvest Control Screen

Note: **EC Cycling's timing and Timed Batch Cycle time are distinct from one another, and they operate independently from one another.**

Override Controls

In the lower left portion of the keypad, four keys are grouped vertically under the title Override Controls: **PUMPS**, **GAS**, **E.C. CYCLE**, and **TEMPERATURE**. They may be used anytime in Idle, Setup, Inoculate, or Running mode. Overrides must be manually activated and cannot be set to automatically turn on or off in advance.

Once active, overrides remain in effect—despite whatever activity the Setup, Inoculate, or Running modes require—until the override is manually cleared (cancelled). For this reason, use overrides carefully until you are very familar with the operation of the *MAXIMIZER* and be sure to clear them as soon as possible!

Pumps: Press **PUMPS**.to display the following screen:

Figure 5-18. Pumps Override Control Screen

The abbreviations used here are the same as the ones used on the Monitor screen (page 5-3). To activate a Pump rate override, use ⬅⬆➡⬇ to move the cursor until it is in the field with the desired pump rate value, press the desired number keys (the desired pump speed), and press **ENTER**.

Overrides are noted by the display of an asterisk (*):

- To the left of the pump label, as shown for F3 pump in figure 5-18.
- To the right of the pump label, as shown for F3 pump in figure 5-1.

The Pumps LED does not turn on when **PUMPS** is pressed (when no pump override is already active). The LED turns on only when a Pump override speed is selected and **ENTER** is pressed. The Pumps LED remains lit until all pump overrides have been cleared.

To clear a Pump override, press **PUMPS**, use ⬅⬆➡⬇ to move the cursor until it is on the desired **asterisk** (not the pump rate value), then press **CLEAR**. The pump may stop or turn, depending on whether Inoculate or Running is active and what their process control settings are at this time.

Gas: Press **GAS** to display the following screen:

Figure 5-19. Gas Override Control Screen

The Gas # shown in this screen is a relative value and the current one in use to control pH. It represents a variable mixture of air and CO_2 being delivered to the GEX (see chapter 6, **pH Control**). When Running or Inoculate is active and the Gas # is not overriden (no asterisk is displayed), the Gas # automatically changes with the demands of the pH control strategy and set point.

Manually overriding the Gas # results in disabling automatic pH control. The gas mixture remains constant because the Gas # remains constant. To override the Gas # press **GAS**, use the number keys to enter a number from 1 – 14, and press **ENTER**. An asterisk (*) appears as shown above, and the Gas LED turns on to indicate a gas override is active. Now, even if automatic pH control wants to vary the Gas # to maintain pH at the desired setpoint, the Gas # will not change.

Why control pH manually via a gas override? If the pH probe in the cultureware has malfunctioned mid-run, automatic pH control will not work. Rather than aseptically replacing the probe, enter a gas override #, wait ~20 minutes, aseptically sample IC medium, assay pH off-line, and adjust the Gas # up or down, if necessary, to achieve the desired pH. Repeat these steps every few days!

If a gas override is a number that is outside of the range set in Process Control, an alarm occurs, but this gas override is allowed. After the override is cleared, the gas # returns to the range set in Process Control.

Notes:

1. **Using gas overrides inhibits pH control and generally should not be used long-term if the pH probe is properly functioning.**

2. **The Monitor screen does not indicate a gas override is active. You must rely on the Gas LED's status — if it is lit, a gas override is active!**

3. **Gas numbers 0 and 15 may be set at any time, even during an active culture run. These numbers correspond to no gas flow and 100% carbon dioxide, respectively (see Figure 6-1) and should be used only at the direction of Biovest technical service personnel.**

E.C. Cycle: Press **E.C. CYCLE** to display the following screen:

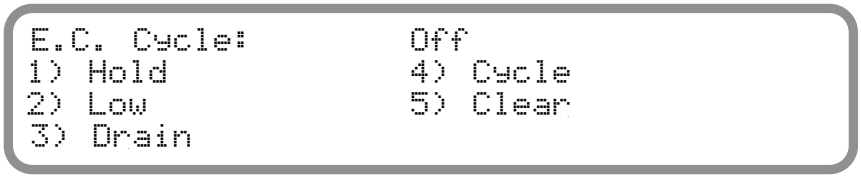


Figure 5-20. E.C. Cycle Override Control Screen

These override functions are related to the fluid movement (cycling) between the IC and EC. Press numbers 1 – 5 activates the relevant menu items immediately without the need to press **ENTER**.

Items 1 – 4 are the options to override EC Cycling. Activating one of these four options turns on the E.C. Cycle LED. Item 5 is used to clear the EC Cycle override (instead of needing to press **CLEAR**), which also turns off the E.C. Cycle LED.

When items 1 – 4 are selected, the Monitor screen shows an asterisk (*) and message in the Cycle field to indicate which EC Cycle override is active. In the override screen, the word "Off" will be replaced to show the same override selection. After an EC Cycle override has been cleared, this word indicates whether process control is in Cycle or Low mode.

The Hold override (item 1) should not be confused with the red **HOLD** key. They perform different functions! Pressing **HOLD** stops all process control and override activities. The EC Cycle Hold override interrupts only the current Rise or Fall phase of the EC Cycling process. When the Hold override is chosen, the level of medium in the Expansion Chamber is maintained at the level when the Hold override became active. The level will not change until the Hold override is cleared.

The Low override (item 2) works similarly to the Hold override, except that the Low override first forces the level in the Expansion Chamber down to its low volume (108 ml), then the level is maintained at this level until the Low override is cleared.

The Drain override (item 3) is only shown when the Idle mode is active. This override is provided for use afte the culture run is terminated. When the Drain override is chosen, pressure is applied to the Expansion Chamber to force **all** of the media out of the EC Chamber and down the Cell Removal line to collect the product into syringes. If collecting the product is unnecessary, leave the Cell Removal clamp closed, and the media is then pushed into the IC circuit. This override merely drains the flowpath before cleanup operations at the end of a run. Refer to Section 6, **End of Culture Procedures**, before using this feature.

Note: **If cell buildup is excessive, completely draining the EC Chamber using the EC Cycle override may not work. Any medium remaining in the EC Chamber may be removed by using the Cell Removal line, even though product collection is unnecessary.**

The Cycle override (item 4) activates the EC Cycling (Rise/Fall) process using the parameters currently set. This allows EC Cycling to function even during the Idle, Inoculate, or Setup mode, when EC Cycling is normally disabled. In these modes EC Cycling occurs using the default parameters (70 ml, 15 minute Rise, 15 minute Fall). If Running mode is active, the Cycle override uses these same default parameters when the Elapsed Time is less than the EC Cycling Delay time. Otherwise, the Process Control parameters are used when the Elapsed Time is greater than the EC Cycling Delay time.

Temperature: Press **TEMPERATURE** to display the following screen:

Figure 5-21. Temperature Override Control Screen

The temperature below "Current" is the current temperature that is measured from the temperature probe that is installed in a fitting in the cultureware in the incubator.

The temperature below "Set Point" is the current control value **in effect**. This means that in the Running mode, the temperature setpoint used by the instrument will be the one set in Process Control, unless a Temperature override is active. In all other modes (Idle, Setup and Inoculate), the setpoint is 37°C unless a Temperature override is active.

When a temperature override is used, an asterisk (*) is displayed to the left of the word "Set Point", and the Temperature LED will be turned on. An asterisk also appears on the Monitor screen next to Tp.

To activate a Temperature override, use ⬅⬆➡⬇ to move the cursor to the value under Set Point, press the desired number keys (the desired temperature), and press **ENTER**. The acceptable range of values is 24.1–45.8°C in increments of 0.1°C. To remove the Temperature override, use ⬅⬆➡⬇ to move the cursor to the asterisk and press **CLEAR**. After the override has been cleared, the value below "Set Point" returns to the current setpoint value.

NOTE: If the Temperature override is set to a value outside of the alarm limits in Process Control, an alarm will occur when the temperature exceeds these limits.

NOTE: A temperature override may not be able to attain the desired value. This limitation is bound by the temperature of ambient air and the temperature and flow rate of incoming media. Contact Technical Services for further details.

CALIBRATE Key

Press **CALIBRATE** to display the program and onscreen instructions to calibrate the pH and DO probes. Calibration can be done at any time during an active culture run.

Calibrating either probe requires offline analysis of an IC sample of medium that is aseptically removed from the cultureware. See chapter 6, **Probe Calibration**, for further details.

HOLD Key

Pressing **HOLD** results in the following screen:

Figure 5-22. Hold Key Selection Screen

Use the Hold feature to respond to an imminent or on-going crisis or to terminate the instrument's current state (during SETUP, for example) or the active culture run. An example of a crisis is a media leak from a tubing line.

Pressing **HOLD** stops these functions whether they're operating from process control or override settings, where applicable:

- All pumps stop
- pH control stops
- Oxygenation stops
- EC Cycling control stops
- Alarm monitoring stops
- Logging stops

If **HOLD** was pressed to respond to a crisis after the cultureware has been inoculated, it is important to resove the problem and resume normal operation as quickly as possibe to minimize the negative effects of no pumping, no pH control, etc. to the cell culture.

After resolving the problem and to resume normal operation, press **1**.

To terminate the instrument's current state or the active culture run, press **2**. The following changes occur:

- IDLE state is activated
- Elapsed time resets to 0–00:00
- Process control settings are reset to the default values
- Active overrides stop and these settings are reset to the default values
- All six items listed above stop

After pressing **2**, alarm messages remain in queue and must be reset manually. Incubator temp. control continues to function before and after pressing **1** or **2**.

NOTE: Special use of HOLD during the Fill/Flush procedure and Pressurization test is described in chapter 6.

ALARM Key

When the Alarm LED is off, no alarm is stored in the alarm queue. In this situation, pressing **ALARM** displays the following screen:

```
No errors reported


Press ENTER to continue.
```

Figure 5-23. Alarm Screen — No Alarm

When the Alarm LED is on (and the alarm screen is not displayed), an alarm(s) is stored in the alarm queue. In this situation, pressing **ALARM** displays a screen specific to one type of alarm. An example alarm screen:

```
pH out of range
 13 0 0                    TIME:    0-00:00
← PREVIOUS ALARM              NEXT ALARM →
       PRESS ENTER TO RESTORE THIS ALARM_
```

Figure 5-24. Alarm Screen — Example Alarmt in Queue

Press ⬅ or ➡ to review however many alarm messages are stored in the queue. Alarms occur when: a) probes in the cultureware or instrument measure a value that is out of range or b) instrument hardware circuitry malfunctions.

MAXIMIZER recognizes 33 distinct alarms, most of which are active only during INOC and RUNNING modes. When an alarm occurs, the Alarm LED turns on, the alarm message is sent to the printer or ACULINK-DATA LOG, and the audible alarm and external alarm relay are activated.

Press **ALARM** to silence the audible alarm and deactivate the external alarm relay. The display changes to show the most recent alarm message (if more than one alarm is activated).

Each time a new alarm occurs you have two choices to respond:

- Press **ENTER** to restore (re-enable) a particular alarm:
  For example, a pH out of range alarm occurs. Press ⬅ or ➡ to display this particular alarm, if necessary. When this alarm is displayed, pressing **ENTER** restores this alarm only. If other alarm(s) are stored in the alarm queue, they must be restored individually. Press **ENTER** only when the monitored parameter, pH in this example, is no longer out of range. Otherwise, another pH out of range alarm will recur in a matter of several seconds.
- Leave the alarm message stored in alarm queue (prevent its recurrence):
  Do this when the monitored parameter, pH in this example, remains out of range. In this case, press **MONITOR** while the problem is examined. Once pH is no longer out of range (is within the alarm limits set in **PROCESS CONTROL**), restore the pH alarm by following the instructions above. If this is not done and pH goes out of range again in the future, the pH alarm will not be recognized, which prevents what happens when an alarm occurs (described on the previous page).

The Alarm LED remains lit when at least one alarm message is stored in the alarm queue. Storing the alarm message continues to silence the alarm and allows you to perform normal activities while also evaluating what may have caused the alarm.

When an alarm is stored in the alarm queue and the Alarm LED is on and the audible alarm is silenced: when a new alarm occurs that is not stored in the alarm queue, the audible alarm and external alarm relay will activate. Press alarm to silence the audible alarm and deactivate the external alarm relay. Respond to this new alarm according to the same two choices described above.

There are a few critical alarms that monitor the instrument's internal circuitry. If one of these alarms occurs and is then stored in the alarm queue, subsequent occurrences of the same alarm will nevertheless cause the audible alarm and external alarm relay to be re-activated. Inform Biovest's Account Services of this situation!

When the last message in the queue has been restored, the screen in figure 5-23 appears. Press **ENTER**. The Alarm LED turns off and the Monitor screen appears.

Note: **Chapter 7, Troubleshooting, lists and describes all available alarms.**

Diagnostics Key

A special key exists to permit access to a series of four screens that display information that is automatically monitored by the instrument. Most of this information is used by and meaningful to trained service personnel.

One of the diagnostic screens (shown below) is routinely used to test the pH and DO probes prior to autoclaving them for use in a culture run. The pH probe is tested by assessing its A/D value (126 as shown below). The DO probe is tested by assessing its A/D value (150 as shown below). A/D values represent the electrical signal from the probes and are reliable means of evaluating the probes' functionality. Operational details of this testing are provided in chapter 6, **pH Probe Validation** and **DO Probe Validation.**

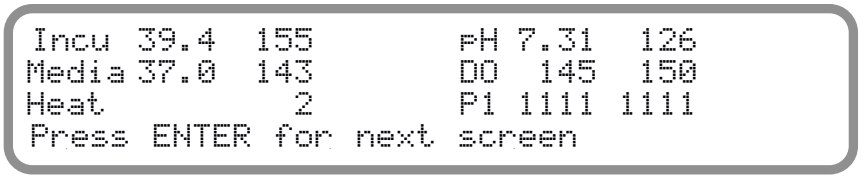


Figure 5-25. Diagnostics Screen 0 for pH and DO Probe Validation

Press **START** then **HIDDEN KEY** (see figure 5-1) to display whichever of the four diagnostic screens was last viewed. Press **ENTER** to scroll through the four diagnostics screens and find the one you want.

Press **MONITOR** at any time to exit from any of the Diagnostics screens.

Chapter 7, **Troubleshooting**, provides details of the diagnostic screens.

PROCESS CONTROL STRATEGY

You may wish to review Section 1, **System Theory**, before proceeding through this section. Here, the overall operating procedures of the ACUSYST-*MAXIMIZER* are explained with emphasis on pH control, dissolved oxygen delivery, media feed strategy, and harvesting technique. Then an outline is presented to show the basic sequence of activities of a typical culture run. This is followed by detailed instructions on each item in that outline, so you will be able to set up, inoculate, and operate a culture run in the *MAXIMIZER*.

pH Control

MAXIMIZER actively controls pH to maintain the user-defined setpoint when in RUNNING mode. pH is monitored on-line (using the pH probe in the cultureware) and controlled in real time, with a 10-second sampling and correction interval.

MAXIMIZER uses three techniques to control pH, described below. The primary method, Gassing, remains in use throughout the active culture run (except for briefly stopping during the inoculation phase) even when either or both of two optional, supplemental methods of controlling pH are enabled and in use.

- **Gassing:** pH is primarily controlled by delivering variable gas mixtures and flow rates through the gas exchanger (referred to as "gassing"). $\%CO_2$ increases when pH is above its setpoint. $\%CO_2$ decreases (or air flow rate increases) when pH is below its setpoint.
- **Base Addition (Optional):** Factor 1 Pump pumps an alkaline buffer solution to counter the effects of lactic acid. The recommended base solution is 0.1 – 0.5 N NaOH or 8.5% $NaHCO_3$ in distilled water.
- **Medium Dilution (Optional):** Media Pump pumps additional medium to dilute lactic acid.

See chapter 5, **Factor 1 (F1, Base Addition) Pump** and **Medium Pump Rates Rate** for further information about Base Addition and Medium Dilution.

Gassing's variable gas mixtures are assigned relative *Gas Numbers* that range from 0 – 15. Only 1 – 14 are used to control pH during an active culture run. See Figure 6-1. Gas # 1 is the most potent for raising pH. Gas # 14 is the most potent for lowering pH. (Gas #15 is used by service personnel only.)

In RUNNING mode, Gas #s 1 – 14 automatically change as necessary to respond to changing culture conditions and maintain pH at its setpoint.

In IDLE, SETUP, and INOC modes, Gas 0 is used.

GAS NUMBER	RELATIVE AIR FLOW RATE	PERCENT AIR	PERCENT CO_2
0	0	0	0
1	7	100	0
2	6	100	0
3	5	100	0
4	4	100	0
5	3	100	0
6	2	100	0
7	1	100	0
8	7	97	3
9	6	96	4
10	5	95	5
11	4	94	6
12	3	92	8
13	2	89	11
14	1	80	20
15	0	0	100

Figure 6-1. CO_2 Concentration and Air Flow Rates

Note: **Gas 15 is used only by service personnel.**

User-Defined pH Control Parameters:

The following parameters are set in their Process Control screens:

- pH setpoint
- Allowed range of gas numbers
- Medium pump rate for Medium Dilution
- Base (F1) pump enabled/disabled for Base Addition
- Base volume to be pumped before start of Medium Dilution

Automatic gassing system control and pH control can be disabled by overriding Gas #, Media Pump speed, or F1 Pump speed.

Overview of pH Control Algorithm:
The following pH control algorithm functions when no overrides are active for Gas # or the MP and F1 pumps. These overrides take precedence over calculations made by the pH control algorithm and partially or completely disable pH control.

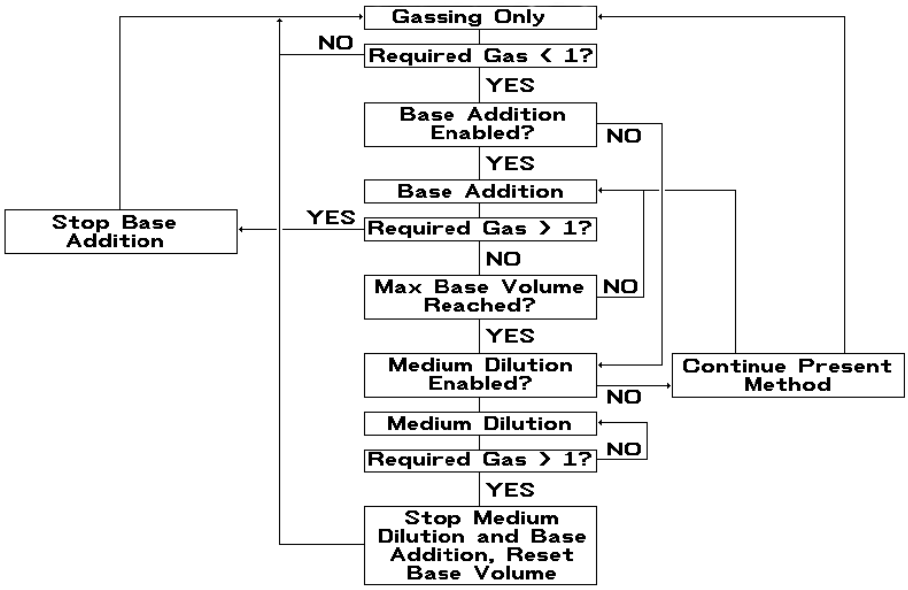


Figure 6-2. Flowchart of pH control algorithm

Gassing:
The pH control algorithm uses a PID controller to automatically determine and adjust the required gas mixture (variable $\%CO_2$ + air) and air flow rate. pH control preferentially uses this gas mixture and flow (referred to as *Gassing*), but supplements it with base addition and medium dilution, which may be individually enabled. Gassing is always in effect, even when these supplementary methods of pH control are used.

In Process Control the user sets the allowed range of gas numbers (`GAS #: LOW` and `HIGH`). Default `LOW` is `1`. Default `HIGH` is `14`. As pH drops, `Gas #` automatically drops to compensate. `Gas #` continues to drop as pH continues to drop. Once `Gas #` reaches the `LOW` value, it cannot drop further. If pH now drops below its setpoint, the algorithm advances to Base Addition. If Base Addition is disabled, the algorithm advances to Medium Dilution. If Medium Dilution is also disabled (`Dilution` rate < `Basal` rate), the algorithm continues to use only Gassing to adjust pH. pH may slowly drop, which often is not detrimental to the culture, unless it drops to a very low level, which occasionally happens.

Base Addition:
Base Addition is optional and disabled by default. Base Addition may be enabled or disabled, as desired, throughout the active culture run via its Process Control screen.

Enable or disable Base Addition in its Process Control screen. If enabled, also enter the desired Base Volume before start of Dilution. that is to be pumped into the system before Medium Dilution is activated.

When gassing alone cannot keep pH at its setpoint (current Gas # = the LOW value) and Base Addition is enabled, the F1 Pump automatically starts pumping base at ⅒ of the Media Pump's rate or 5 ml/hr, whichever is greater. F1 Pump continues to pump base until the desired Base Volume before start of Dilution has been pumped, as long as pH remains below its setpoint. At this point, if pH is still below its setpoint, Base Addition continues (F1 keeps pumping base) and Medium Dilution is also activated, if it is enabled.

If pH returns to its setpoint (and the current Gas # ≥ the LOW value) before the desired Base Volume before start of Dilution has been pumped:

- F1 Pump stops
- Base Volume before start of Dilution is reset to the entered value
- Gassing alone controls pH

If pH again falls below its setpoint and Base Addition is activated, the previous amount of base that was pumped is forgotten and the user-defined volume now can be pumped again before Medium Dilution is re-activated, if it's enabled.

Base addition is usually unnecessary for most applications and may be left disabled. However, if pH is dropping more than seems safe, you may wish to enable Base Addition to be prepared and prevent pH from dropping too far and jeopardizing culture viability. Prepare a sterile bottle of base solution, aseptically connect it to F1 tubing line, and open the F1 clamp. Then, if Base Addition is needed, it is ready to be automatically pumped.

Medium Dilution:
Medium Dilution is optional and disabled by default. Medium Dilution may be enabled or disabled, as desired, throughout the active culture run via its Process Control screen.

Medium Dilution is:

- Enabled when Dilution rate > Basal rate
- Disabled when Dilution rate < Basal rate

When Medium Dilution is enabled, it is activated for either situation:

- Base Volume before start of Dilution has been pumped, current Gas # is the LOW value, and pH remains below its setpoint.
- Base Addition is disabled, current Gas # is the LOW value, and pH drops below its setpoint.

When Medium Dilution is activated, the result is the Media Pump speed automatically increases from the Basal rate up to the Dilution rate to dilute Lactate (an acid) in the cultureware in an attempt to reduce pH to its setpoint.

Medium Dilution continues until pH reaches its setpoint and the current Gas # ≥ the LOW value. Medium Dilution then stops, and the MP speed automatically decreases from the Dilution rate to the Basal rate. Only Gassing continues to be used to control pH. If pH drops below its setpoint again, the whole cascade of using Base Addition and Medium Dilution repeats as described above.

When Medium Dilution is disabled, the MP speed remains at the Basal rate, so no extra medium is used to dilute lactate.

Oxygen Delivery

Regardless of the gas mixture number used for pH control (see above), the GEX provides near-saturation levels of dissolved oxygen (DO) in the medium before it reaches the BRX(s). As a culture matures, cells will consume oxygen from the surrounding medium, and produce a lower DO value downstream from the BRX(s). The cultureware design allows you to monitor the DO of medium either before or after it is pumped to the BRX(s). The difference between these values is used to calculate an oxygen uptake rate (OUR) for the culture (see Appendix E). Equally important is the post-BRX DO value itself because it represents the lowest level of oxygen to which the cells may be exposed. This may be used as a guideline for DO delivery to maintain a healthy culture.

DO delivery is regulated by the speed of the circulation pump. Therefore, as the post-BRX DO value falls due to cellular activity, the circulation pump rate can be increased to maintain a desired post-culture minimum level of DO. You may wish to consider maintaining different post-BRX DO values for the growth and production phases of a *MAXIMIZER* culture.

Feed Strategy

The most commonly used feeding strategy in perfusion is based on providing nutrients (mainly glucose) or diluting wastes (mainly lactate). In the case of nutrients, a minimum concentration (setpoint) of glucose is established in advance of setting up the culture, and the feed rate is manually adjusted to ensure that this level is attained by the next sampling period. A similar maximum setpoint may be established for lactate, so that an upper tolerable concentration is known. Regardless of which metabolite is considered more important, the *MAXIMIZER* medium rate must be changed manually to maintain the desired level of nutrients and/or wastes. A regular schedule of sample removal and analysis for metabolite concentrations provides important data for this determination.

Note: **Biovest International offers a software package (ACUDATA) for IBM-compatible computers. It is based on a non-linear regression growth rate formula. Use ACUDATA during a culture run to calculate media pump rates that will maintain metabolite concentrations in the future based on recent nutrient consumption rates, which ACUDATA also calculates. See Appendix B of this manual for information and contact Account Services for more details.**

Special consideration must be placed on any labile components of the medium. Because glutamine is known to degrade spontaneously to ammonia, especially with increasing temperatures, it makes for a prime example. You may choose to formulate your basal medium without glutamine, and make a concentrated glutamine factor solution. If so, Process Control or Override Controls may be used to pump this factor into the flowpath at a rate which effectively delivers glutamine as if it were incorporated in the basal medium. (EXAMPLE: If your basal medium normally has 4mM glutamine, you can prepare a 160mM glutamine factor, and set Process Control or Override Controls to add it at ¼₀ of the basal medium rate.) The advantage of this technique is to allow you to store such an unstable component in a small volume which can be chilled in an ice bath to prevent its breakdown.

Note: **Although Biovest International offers bottles up to 10 liters in size, you may feel that larger containers are necessary for weekends and/or total feed rates greater than 400 ml/hr (9.6 l/day). Consult Account Services for alternative sources of vessels and their appropriate sterilization conditions.**

Harvesting Strategy

The ACUSYST-*MAXIMIZER* may be programmed for several types of harvesting protocols. You must decide which method is best suited for harvesting the secreted product from your cell line. Continuous harvest is the most common technique. The two other options available through Process Control include harvesting at **every** low Expansion Chamber volume or, each low Expansion Chamber volume that occurs after a user-defined number of EC Cycles that are skipped. Of course, the **PUMPS** Override Controls allows you the flexibility to perform batch harvesting by overriding the HP manually to collect the desired volume regardless of the phase of EC Cycling (see Section 5, **User Interface**).

Be aware that removing fluid from the ECS is not a selective process. All material in the ECS will be pumped out, including serum, secreted protein, and loosely adherent cells. For this reason, the early stages of harvesting should be complemented with an equivalent replacement rate of serum factor.

As the culture matures, the harvest rate (or frequency) may be increased gradually until the daily yield or concentration of product has reached a peak. Then, because dense cultures frequently need less serum, the serum factor pump concentration may be lowered gradually to further optimize production. The key to both of these strategies is to effect changes slowly to avoid sudden shifts in environmental conditions which might cause an adverse reaction by the culture.

OUTLINE OF A CULTURE RUN

The following list shows the major events that occur in a typical culture run. They are presented chronologically, but due to the variability of each *MAXIMIZER* owner, the chart cannot show the length of time required for each step to be completed. Each step is described in detail.

Milestones For A Culture Run

Biological Setup Procedures
- Cell line characterization
- Sterilization of bottles and cap assemblies
- Preparation of media
- Scale-up for inoculation

Instrument Setup Procedures
- Instrument checkout

Cultureware Setup Procedures
- Probe validation
- Sterilization of probe assemblies
- Completion of flowpath module
- Flowpath installation in *MAXIMIZER*
- Flowpath pressurization test
- Fill/Flush procedure

Maintenance Procedures
- Sampling
- Calibration
- Replacing bottles
- Replacing luer-activated valve or septa
- Removal of cell debris

Pre-inoculation Procedures
- pH calibration
- DO calibration
- Cycling
- Osmolarity
- Offgassing
- Cytotoxicity testing
- Sterility testing
- Serum pre-treatment of the ECS

Inoculation Procedure
Post-inoculation Procedures
End of Culture Procedures

BIOLOGICAL SETUP PROCEDURES

Cell Line Characterization

Successful mammalian cell culture production requires the capability of precise process control. The *MAXIMIZER* is equipped with a process control system which provides you with complete control of the cell environment. A good process control strategy will allow cells to grow to a high density quickly and then shift control parameters to keep the cells in a stable environment with production at its peak. It also allows you to vary conditions, if required, to maximize that production.

Thus, a successful process control strategy depends on a thorough knowledge of the characteristics of your cell line. There are many possible methods for characterizing cells, but they all should establish specific values for:

- optimum pH
- choice of medium
- serum dependence
- glucose and lactate levels
- product yield
- cell viability

These established values and other information are used to obtain target values for the process control strategy. Before beginning production, a full characterization of your cell line should be completed.

All cell lines differ in their requirements, and a thorough knowledge of your cells is important. For further information on establishing process parameters, contact Account Services.

Sterilizing Bottles and Cap Assemblies

All bottles and cap assemblies used in the *MAXIMIZER* system must be sterilized to minimize the potential for system contamination. The time required for complete sterilization should be determined by autoclave validation.

All bottles, except those being used for outflow and harvest, should be sterilized with their original caps on to facilitate the sterile addition of media and/or factor to the bottle. The special delivery cap assemblies should be sterilized separately, wrapped in aluminum foil or placed in an autoclave bag.

Note: Because instruments and conditions may vary, ensure sterility by validating your autoclave before carrying out this procedure.

Note: Filters on the cap assemblies may be autoclaved up to five times.

1. Validate your autoclave.
2. Clean and wash bottles, caps, and cap assemblies using a tissue culture grade soap and water. Use good laboratory procedures for cleaning tissue culture glassware.

Note: Prior to washing cap assemblies, remove the filter and two Luer plugs. A syringe may be used to clean the IN and OUT ports on the cap assemblies. Do not wash the filters.

Note: The plastic pouring rings should be removed from the bottles and washed separately.

3. Replace the pouring rings on all bottles. Put the caps on all bottles, leaving them loose enough to allow steam to enter. Cover these caps with foil or other autoclavable covering.
4. Put the two male Luer plugs in their respective holes (IN and OUT ports) on the cap assemblies. Install a 0.2 micron Acrodisc filter onto each cap assembly. (These are supplied as part of the Bottle and Cap Startup package. See Appendix B.). Wrap the cap assemblies with foil or other autoclavable covering.

5. Place all prepared bottles and cap assemblies in the autoclave.
6. Autoclave at 121°C at 15 psi maximum for approximately 30 minutes, or at the conditions determined during your autoclave validation.
7. Remove the bottles and cap assemblies from the autoclave and allow them to cool. Tighten caps when completely cool. Do **not** remove the covering.
8. Store bottles in a dust-free cabinet until you are ready to use them. Bottles may be stored sterilized for six weeks before they need to be re-sterilized. It is a good idea to sterilize extra bottles so that they will be available as needed. This can save time during your production run.

Note: **Each bottle is equipped with a removable plastic pouring ring. Although it may be autoclaved at the conditions described above, it will not withstand the dry heat conditions used in depyrogenating glassware. If you employ this process, you must remove the pouring ring before placing the bottle in the depyrogenating oven.**

Medium Preparation

Be aware that it can take up to 6 – 8 weeks for sufficient medium for the run to be ready, so plan the date to inoculate the *MAXIMIZER* accordingly.

The medium should meet any special requirements suggested by your cell line characterization, and can be pre-packaged or made in the laboratory. The medium must contain all of the necessary nutrients, adequate sodium bicarbonate buffer, and any required pH indicators or antibiotics. The choice of a medium is usually determined by cell requirements, the user's experience, and financial considerations.

Note: **A high glucose medium (3.0 – 4.5 g/l) is recommended for use in the MAXIMIZER to minimize the amount of medium required while supplying adequate glucose to the cell culture. A glucose level lower than 3.0 g/l may result in excessive medium usage.**

Note: **pH control in the MAXIMIZER is based on a bicarbonate buffering system. We suggest 3.5 g/l of Sodium bicarbonate when using approximately 4 g/L glucose. At least 2.2 g/l of Sodium bicarbonate should be present in the medium. Other pH buffers such as HEPES or Tris should NOT be used. If glucose concentrations are higher than suggested, it may be necessary to increase the concentration of $NaHCO_3$ to prevent problems with pH control.**

Procedures for medium preparation will vary depending upon the medium being prepared. Use the procedure most appropriate for your circumstances.

Sterilizing Medium

As one of the key components of the system, the growth medium used must be sterilized before it is pumped into the system. Once sterilized, medium can be transferred into sterile bags or bottles.

Medium Quality Control

The medium used in your system is one of the key ingredients to a successful experiment or production run. If it is in any way contaminated, the entire run may be at risk of contamination. Thus, you should always check your medium for contamination before pumping it into the system.

Because medium quality control requires one week to complete, it is important to complete medium checks not only before beginning, but also throughout your production run. Several quality control methods can be used to check the quality of your medium.

If medium has been prepared and stored for over six weeks before being used, it should be tested to be sure that it is still usable. Certain nutrients in the medium may degrade during a long storage period. If there are any questions regarding medium quality, please refer to the manufacturer's recommendations or contact Biovest International's Account Services.

Scale-up for Inoculation

Before you proceed to **Instrument Setup Procedures**, you should begin the scale-up preparations for inoculating the *MAXIMIZER*. As there is no single method suitable for all cell lines, this manual will not recommend specific procedures. Typical protocols for growing cells in T-flasks, spinner flasks, or roller bottles are probably already in place at your facility. Use them to prepare the inoculum so that the inoculation can proceed smoothly. Account Services and the opening paragraphs of the **Inoculation** part of this section will be able to provide you with the necessary background information.

INSTRUMENT SETUP PROCEDURES

Prepare Utilities

1. Regulate the two gas supplies to 15 ± 1 psi
2. Open the CO_2 (required) supply to the *MAXIMIZER*
3. Open the Backup Air (optional) supply to the *MAXIMIZER*

Prepare Data Logging Equipment

If ACULINK-DATA LOG is in use:

1. Connect the serial cable to the computer's serial port and the **PC INTERFACE** port of the ACULINK Instrument Interface Module.
2. Connect the parallel cable to *MAXIMIZER* and any one of the four **ACUSYST** ports of the ACULINK Instrument Interface Module.
3. Plug the power supply into the ACULINK Instrument Interface Module.
4. Turn on the computer and start the ACUSOFT-Link program.
5. To enable exporting the run log to ASCII format for use in various other computer programs, from within the ACUSOFT-Link program press **SHIFT F2** and set the parallel port to ⎚.
6. Further setup and operation details are provided in the operations manual for ACULINK-DATA LOG.

If a printer is in use rather than ACULINK-DATA LOG:

1. Load paper
2. Plug in the parallel (or serial) cable to the printer and the *MAXIMIZER*
3. Turn on the printer's power
4. Configure the printer to be *on-line* or *selected*, depending on printer model. Consult the printer's user manual for further information.

System Checkout

Before starting any culture run, the following checkout list should be consulted, and the appropriate procedures should be performed to ensure that everything is in working order. If you have a certified service technician who can inspect the internal electronics and gassing system, you may consult him/her before performing the following checkout.

Pre-Culture Checkout List

1. System Tests
2. Instrument Pressurization Test
3. Validation of pH Probe and DO Probe
4. Pressure Testing of pH and DO Probe Case Assemblies

Note: **The pH and DO probes should be tested for satisfactory performance before installing them in the flowpath. This testing is done prior to installing the probes in their cases, which occurs before pressure testing and autoclaving the probe case assemblies. Contact Account Services if you have questions about a probe or cable.**

Note: **Skip steps 3 and 4 for the sterile, disposable pH probe, see page 6-24, pH Probe Selection and Appendix B, Ordering Information.**

System Tests:

These tests and the one titled **Pressurization Test** should always be performed **before** probes are tested and a new cultureware is opened and put into use.

Plug in the *MAXIMIZER* and turn on its power switch at the rear of instrument.

The LCD should show `IDLE` in the upper left corner. If not, press **HOLD** then **2**.

Press **SET-UP** to display the `System Tests` menu.

Figure 6-3. System Tests Menu Screen

When **SET-UP** was pressed above, the `Logging Interval` screen may have been displayed instead. Its use is described later in this chapter.

Figure 6-4. Logging Interval Screen

Press **ENTER** to display the `System Tests` menu, if necessary.

Note: **Repetitively pressing ENTER cycles between the `System Tests` and `Logging Interval` screens.**

Perform each of the System Tests consecutively by pressing **1**–**6**. After each test passes, press **ENTER**. If a test fails, contact Biovest's Account Services before proceeding with the run.

1) LCD Test:
   a) Press **1**
   b) LCD displays a 4x40 pattern of rectangles
   b) All pixels of each rectangle should be black and easy to see
   c) Cursor flashes in the lower-right corner
   d) If all pixels are black, the test passed
   e) Press **ENTER**

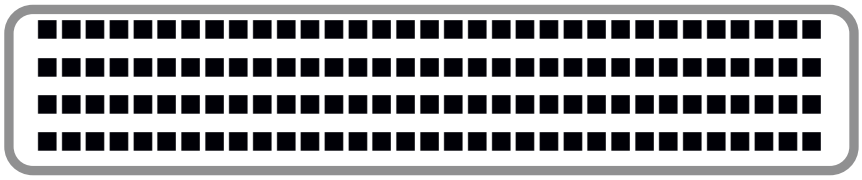


Figure 6-5. LCD Test Screen

2) Alarm Test:
   a) Press **2**
   b) LCD displays AlarM Test:
   b) Audible alarm turns on for several seconds
   c) Audible alarm turns off
   d) If the audible alarm makes noise, the test passed. (This is the sound that occurs whenever an alarm happens during a run.)
   e) Press **ENTER**

Figure 6-6. Alarm Test Screen

Note: **The volume of the audible alarm can be adjusted somewhat by turning its plastic cover to open or close the openings built into the cover. Repeat the Alarm Test (by pressing 2) as often as necessary to allow you to adjust this volume. (The audible alarm is mounted at the rear of the instrument. Refer to Figure 3-3 for further details.)**

3) LEDS Test:
   a) Press **3**
   b) LCD displays LED Test:
   c) If all 13 red LEDs turn on, the test passed
   d) Press **ENTER**

Figure 6-7. LED Test Screen

4) Keypad Test:
   a) Press **4**
   b) LCD displays Keypad Test:
   c) Cursor flashes to the right of this prompt
   d) Sequentially press **1**–**0** and the decimal key
   e) LCD updates to show each number and decimal at the top of the screen and in the lower, right-hand corner
   f) Sequentially press ⬆⬅➡⬇ and **START**
   g) LCD updates to show the name of each key
   h) Press **CLEAR** and note the value in the lower, right-hand corner disappears
   i) If the keys and LCD respond as described, the tests passed
   j) Press **ENTER**

Figure 6-8. Keypad Test Screen

5) Printer Test:
   a) Press **5**
   b) LCD displays Printer Test:
   c) Printer advances paper by **one** page
   d) **Four** lines of various letters, numbers and characters are printed
   e) If steps c and d occur, the test passed
   f) Press **ENTER**

Figure 6-9. Printer Test Screen

Notes:

1. **This test merely checks the communications between the MAXIMIZER and the printer. It does not set the printer log interval.**
2. **This test applies to a printer that is connected either to the serial or the parallel port.**
3. **If the four printed lines are printed on a single line or there is an empty line between each printed line, the printer is not configured properly, which must be corrected.**
4. **This test does not apply to ACULINK-DATA LOG. The MAXIMIZER does not have a built-in method of testing ACULINK-DATA LOG. To test that it is working, perform the following:**

   ACULINK-DATA LOG Test:
   a) Follow instructions on page 6-16 to display Logging Interval screen
   b) Press **1** then **ENTER**
   c) Press **START** and **PROCESS CONTROL**
   d) Press **ALARM** to silence the audible alarm
   e) In ACULINK-DATA LOG, navigate to the correct Instrument Monitor screen that represents this *MAXIMIZER*
   f) After ~1 minute ACULINK-DATA LOG should update and show the *MAXIMIZER* is running
   g) If step f happens, the test passed
   h) Press **HOLD** then **2**
   i) Press **ALARM**. Press **ENTER** until all alarms are restored and the Monitor screen appears
   j) Change Logging Interval to 0 mins

6) PROMS Test:

a) Press **6**
b) LCD displays top screen of Figure 6-10
c) Press **ENTER**
d) LCD displays middle screen of Figure 6-10
e) Press **ENTER**
f) LCD displays bottom screen of Figure 6-10
g) Press **ENTER**
h) If each of the three screens shows the correct software names, part numbers, and revision levels, the test passed
i) Press **ENTER**

```
MP          3232-000   Rev   A
OP          3232-000   Rev   A
HP          3232-000   Rev   A
CP          400146-000 Rev   C
```

```
F1          3232-000   Rev   A
F2          3232-000   Rev   A
F3          3232-000   Rev   A
MONITOR     400133-000   Rev A
```

```
UIF         400129-000   Rev B
Control1    400127-000 Rev A1
Control2    400128-000 Rev A1
```

Figure 6-10. PROMS Test Screens
(Showing Current Information)

Notes: **The PROMS test allows you to check the revision levels of the various Programmable Read-Only Memory (PROM) chips. This information will not change unless the instrument is serviced by Biovest personnel.**

Instrument Pressurization Test:

This procedure checks the integrity of the internal gassing system and should be performed before proceeding with the run to ensure the instrument is in good working order.

Note: **The air filter must be attached to the rear of the MAXIMIZER (see Figure 3-3) at this time.**

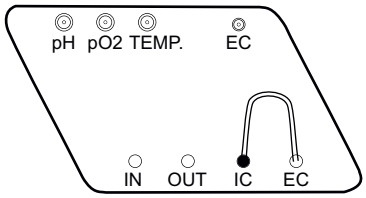


Figure 6-11. Bulkhead preparation for internal system pressure test

1. Use the reusable black-collared Tygon gassing line to interconnect the **IC** and **EC** bulkhead ports inside the incubator (Figure 6-11). This seals the internal gassing system from leaks through the **IC** and **EC** ports.
2. Press **START** then **SET-UP**. The LCD displays the following screen.

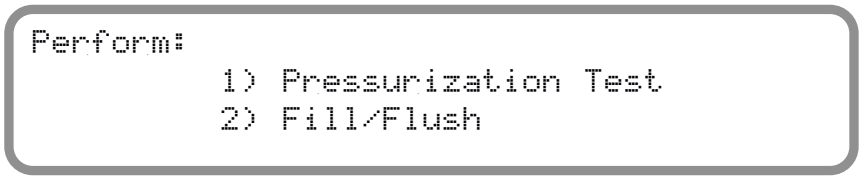


Figure 6-12. Active Setup Menu Screen

3. Press **1** to start the Pressurization Test.
4. Press **ENTER** to begin the test.
5. Instructions for testing a cultureware are displayed in a series of five screens. These screens' instuctions are not relevant for pressure-testing the instrument itself. Ignore the instructions to open or close various clamps in these screens.

6. Repetitively press **ENTER** to advance past these five screens until the top screen of Figure 6-13 appears.
7. The LCD updates to show the three automated stages of the Pressurization Test: `- PRESSURIZING...` , `STABILIZE`, and `TESTING`.

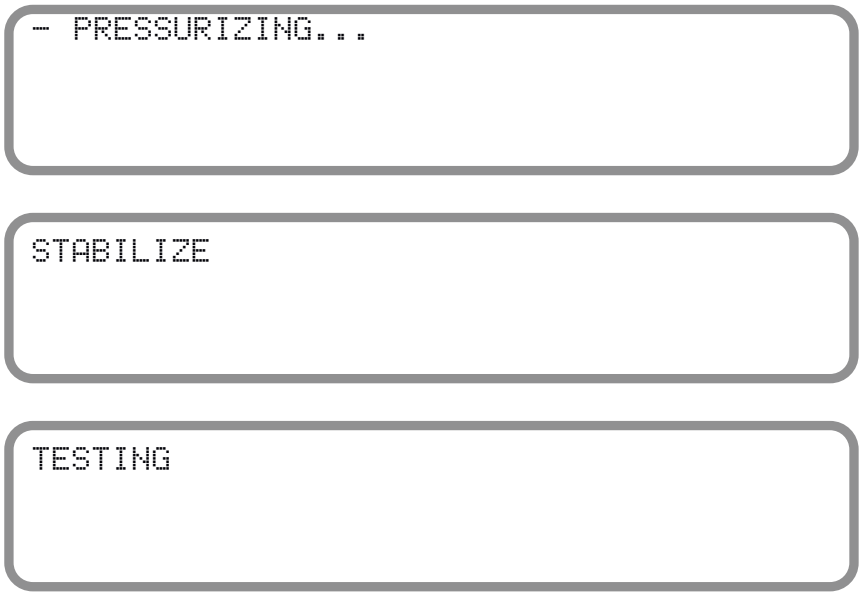


Figure 6-13. Pressurization Test Duty Screens

8. The Pressurization Test automatically finishes within 90 seconds.
   - If there was no leak, the test passes (see top screen of Figure 6-14). Go to step 14.
   - If there was a leak, the test fails (see bottom screen of Figure 6-14). Go to step 9.

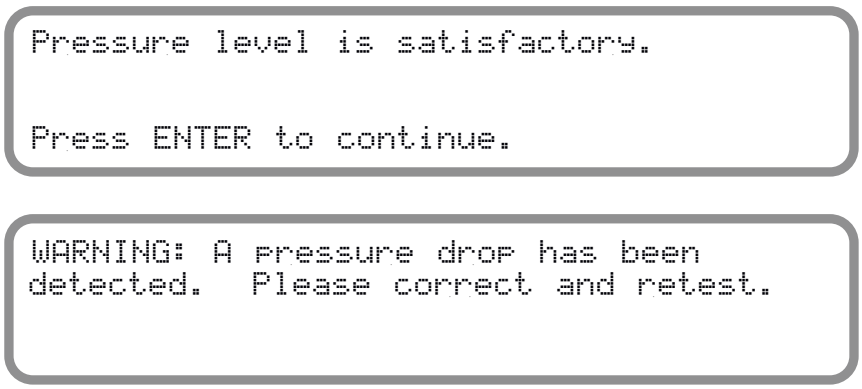

Pressure level is satisfactory.
Press ENTER to continue.
WARNING: A pressure drop has been
detected. Please correct and retest.

Figure 6-14. Pressurization Test Passed Screen and Pressure Test Failed Screen

9. If the instrument Pressurization Test fails, press **ALARM** to silence the audible alarm (and deactivate the external alarm relay).
10. A screen similar to Figure 6-15 appears.

Figure 6-15. Pressuirzation Test Failure Alarm Screen

11. The leak must be located and fixed before proceeding with the run. Potential sources of a leak are:
    a) Tightness of gassing line in the **IC** and **EC** bulkhead ports
    b) Connection of the gassing line's tubing on its male Luer fittings
    c) Damage to the gassing line's male Luer fittings or tubing
    d) Damage to the instrument's bulkhead port female Luer fittings
    e) The instrument has an internal leak
12. For 11 d and e, contact Biovest's Account Services department. For 11 a – c, repair or replace the parts and repeat the Pressurization Test.
13. After each attempt to fix the leak, repeat the Pressurization Test to confirm the leak has been fixed:
    a) If necessary, press **ALARM**
    b) Use ⬅ or ➡ to locate the `Pressure drop detected` alarm
    c) Press **ENTER** to restore the alarm
    d) Press **START** then **SET-UP**
    e) Press **ENTER** if necessary to return to the Active Setup Menu screen (Figure 6-12)
    f) Repeat the instrument Pressurization Test using steps 3 – 8.

14. When the instrument Pressurization Test passes, press **ENTER** to display the following screen.

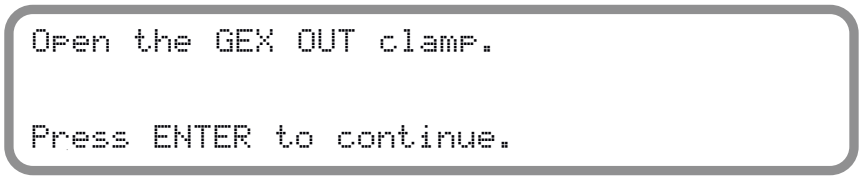


Figure 6-16. Final Pressuirzation Test Screen

15. Ignore the clamp instruction because it is irrelevant when pressure-testing the instrument.
16. Press **ENTER** to return to the Active Setup Menu screen (Figure 6-12).
17. Disconnect the Tygon gassing line from the **IC** and **EC** bulkhead ports. Set it aside for possible use later in the run.

CULTUREWARE SETUP PROCEDURES

pH Probe Selection

The ACUSYST-MAXIMIZER automatically controls pH of the circulating cell culture medium when an in-line pH probe is in use. Biovest offers two types of pH probes for this purpose. One type is reusable. The other type is disposable.

The two types of pH probes are not distinguished by their functionality in controlling pH. Rather, they are distinguished by the expertise, time, testing, documentation, and troubleshooting that are required during their use.

The **reusable pH probe** requires validation, assembly, autoclave-sterilization, and other procedures to prepare it during run startup. Expertise is needed to ensure these steps are properly performed, so the probe functions well and is not damaged. A reusable pH probe has a finite lifespan, however, and will eventually fail. Such failures may not be detected until after the pH probe has been aseptically installed into a cultureware and it contains circulating cell culture medium. Replacement in this circumstance is possible but is time consuming, can lead to contamination, or may negatively impact the cell culture.

The advantage of reusable pH probes is their purchase price is low compared to disposable pH probes.

To proceed with a reusable pH probe, go to the next section, *pH Probe Evaluation*.

The **disposable pH probe** comes as an assembly including the probe, probe case, and electronics cable. Disposable pH probes are provided with a certificate of compliance and are sterile and ready-for-use. Cultureware also are provided in the same manner. Disposable pH probes make run startup and turn-around very fast. The technician obtains one cultureware and one disposable pH probe, aseptically connects them, and starts the run. At the end of the run, the entire disposable pH probe assembly is discarded with the cultureware.

The disposable pH probe has many advantages during run startup and termination. It also eliminates maintenance and storage issues, as well as the risk that a reusable probe will not function during the next production run.

To proceed with a disposable pH probe, skip ahead to *Cultureware Sterile Assembly.*

pH Probe Evaluation

The probe evaluation instructions do not apply to the disposable pH probe.

1. The instrument must be plugged in and turned on.
2. Press **START** then **HIDDEN KEY** to display the diagnostics screens.
3. Press **ENTER** until the following screen appears.
   - 1st column to the right of pH: Standard reading from probe (pH units)
   - 2nd column to the right of pH: A/D reading from probe

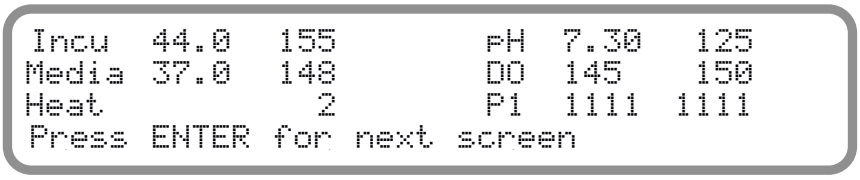


Figure 6-17. Diagnostics Screen 0

4. Remove and save the storage solution bottle and electrical cap.
5. Connect the pH cable to the probe and the bulkhead **pH** jack.
6. Place the tip of the probe in a container with pH 7.0 buffer solution.
7. Place the container and probe so they remain upright without being touched or held. Wait about 2 minutes, then proceed (for the 7.0 buffer test).
8. A functional pH probe's A/D reading in pH buffer 7.0 is 95 – 160 at room temperature. Do not use the probe if the value is outside this range!
9. Remove the pH probe from buffer 7.0, rinse it, and place it in pH buffer 4.0.
10. Repeat step 5.
11. A functional pH probe's A/D reading in pH buffer 4.0 is <3 at room temperature. Do not use the probe if the value is outside this range.
12. Rinse the probe and put on the storage solution bottle and electrical cap.

Notes:

1. **Ignore the readings in pH units during the evaluation. Acceptance is based only on A/D values (not whether pH agrees with buffer's pH).**
2. **Ensure the buffer solutions have not expired.**
3. **If either A/D value is nearly out of range, the subsequent autoclave-sterilization may render the probe unacceptable for continued use.**

DO Probe Evaluation

1. The instrument must be plugged in and turned on.
2. Remove the electrical cap and the protective cap covering the probe's tip.
3. Unscrew the probe's sleeve, remove the probe's membrane cartridge, discard the electrolyte, rinse the probe and membrane cartridge, pour electrolyte into the membrane cartridge without forming bubbles until it is ~¾-full, carefully slide the membrane cartridge on to the DO probe, wipe off electrolyte from the outside of the membrane cartridge, and screw on the sleeve. Ensure no air bubbles are inside the membrane cartridge.
4. Connect the DO cable to the probe and the bulkhead **pO2** jack.
5. Do not allow the DO probe to contact any metal parts inside the incubator. This would not damage the probe, but it would electrically ground the probe to earth ground and prevent it from being polarized.
6. Connect the temperature probe to the bulkhead **TEMP.** jack.
7. Close the incubator door. Allow at least 2 hours for the probe to reach 37°C.
8. In addition to warming to 37°C, the probe receives an electrical charge from the instrument. The electrical charge *polarizes* the probe. The required period of time to fully polarize the probe can vary. Two hours is the minimum suggested time. The best strategy is to allow the probe to polarize overnight before proceeding.
9. Press **START** then **HIDDEN KEY** to display the diagnostic screens, if necessary.
10. Press **ENTER** until the following screen appears.
    - 1st column to the right of DO: Standard reading from probe (mmHg)
    - 2nd column to the right of DO: A/D reading from probe

```
Incu  44.0   155        pH  7.30   125
Media 37.0   148        DO  145    150
Heat           2        P1  1111  1111
Press ENTER for next screen
```

Figure 6-18. Diagnostics Screen 0

11. A functional DO probe's A/D reading when its tip is exposed to air is 65 – 130 at 37°C. Do not use the probe if the value is outside this range!
12. If the A/D reading is unacceptable, disassemble the probe. Use a new membrane cartridge and/or a new bottle of electrolyte.

Notes:

1. **Do NOT touch the membrane cartridge that is at the tip of the DO probe!**
2. **Polarization time is an important step in this procedure. If the A/D count is slowly changing, wait additional time until it has reasonably stabilized before determining if the probe is working within specification.**
3. **Ensure the electrolyte solution has not expired.**
4. **See Appendix B, Ordering Information, for replacement kits of membrane cartridges and electrolyte.**

Probe Case Assemblies

The pH and DO probe assemblies include the two probes and their respective probe cases. The probes are reused as long as they pass the evaluation tests. The probe cases are very durable and last many runs. Only the o-rings should be replaced for each run. New o-rings are included with each new cultureware, so there is no reason to reuse them. Each new cultureware also includes two sets of non-sterile probe case tubing lines used to pressure test and autoclave-sterilize the probe assemblies.

The probe assembly instructions do not apply to the disposable pH probe.

The instructions for assembling the DO probe and reusable pH probe are:

1. Get the o-rings and both sets of probe case tubing lines from the cultureware box. **Do NOT lift the cultureware by its sterile bag if it's necessary to move the cultureware to find these items!** Instead, lift the cultureware by grasping it by its white plastic tray.
2. Remove the o-rings from their bags. They do NOT need to remain sterile now. Inspect the o-rings for damage.
3. Disassemble the probe cases (and ensure they were cleaned at the end of the previous run) and discard the old o-rings, if present. Install the new o-rings and *loosely* thread the lower section into the upper section, see figures 6-19 and 6-20.
4. Connect the threaded pH probe electrical cap finger-tight and connect the DO probe twist-lock electrical cap.
5. Remove the plastic orange cap and sponge from the DO probe tip. Be careful to avoid touching the membrane at the tip of the probe!
6. Insert the DO probe down fully into the case as far as its design allows. This includes gently but firmly forcing the probe tip through the probe case's o-ring. Install the locking ring, but just hand-tight!
7. Insert the pH probe into its probe case. These items have threads. Only make them finger-tight. Do not use tools to tighten them together!
8. Finish tightening the lower sections of both probe cases, but just hand-tight!
9. Slide the DO probe case tubing lines onto the port fittings as far as possible. Either tubing line can go on either port fitting. See CAUTION note below.

10. Connect the pH tubing lines to the pH probe case ports in the following orientation: connect the pH IN/LO tubing line to the lower port fitting of the probe case and connect the pH OUT/HI tubing line to the higher port fitting of the probe case. Follow the next step's instructions to actually connect the pH tubing lines to the probe case port fittings.
11. Rotate the white locking rings to the unlocked position for both pH probe case tubing lines. Press and hold the latch, push the connector fully on to the port fitting, release the latch, and rotate the locking ring to the locked position. Repeat these steps for the other tubing line. Ensure the latches of both probe case tubing lines face upwards.

CAUTION: The pH and DO probe case tubing lines and their connection methods to the probe cases will be leak-free without using cable ties. We recommend to NOT add cable ties to this tubing. Adding cable ties can actually cause leaks to occur!

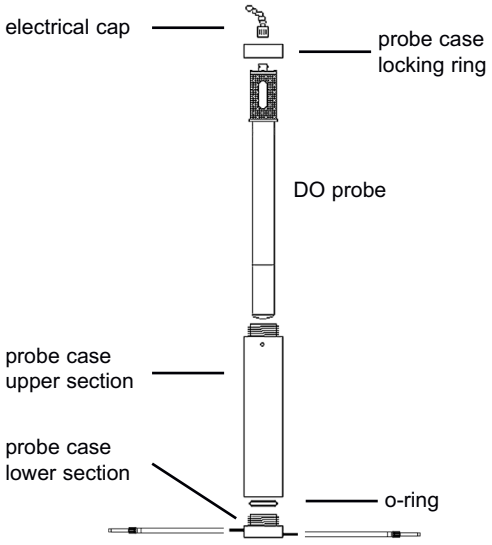


Figure 6-19. Components of DO probe case assembly

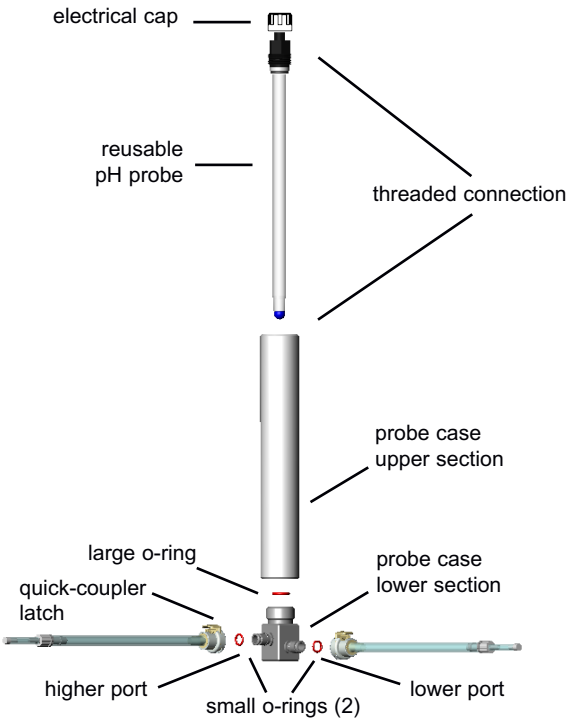


Figure 6-20. Components of pH probe case assembly

Probe Case Assembly Pressure Tests

It is important to pressure-test the pH and DO probe case assemblies to ensure they do not leak. They are tested one at a time, and either probe assembly may be tested first. Successful pressure tests for these probe assemblies ensure that cell culture medium will not leak out at a later time.

The pressure test instructions do not apply to the disposable pH probe.

The instructions for pressure testing the DO probe and reusable pH probe are:

1. Before proceeding, ensure the Instrument Pressurization Test was successful, see page 6-21. If that test fails, the Probe Case Pressurization Tests will fail.
2. Remove the sterility protector (small tubing with white plug) to expose the female Luer fitting from both probe case tubing lines of the DO and pH probe assemblies.
3. Connect the reusable IC and EC gassing lines supplied with the instrument to the **IC** and **EC** bulkhead ports inside the incubator (Figure 6-21).

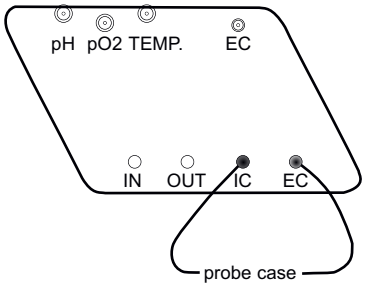


Figure 6-21. Bulkhead Attachments for Probe Case Pressurization

4. Connect the male Luer fittings of the two gassing lines to the female Luer fittings of the probe case tubing lines from step 2.
5. Press **START** then **SET-UP**.
6. Press **1** to start the Pressurization Test.
7. Press **ENTER** to begin the test.
8. Instructions for testing a cultureware are displayed in a series of five screens. These screens' instuctions are not relevant for pressure-testing the probe case assemblies. Ignore the instructions to open or close various clamps in these screens.
9. Repetitively press **ENTER** to advance past these five screens.

10. The LCD updates to show the three automated stages of the Pressurization Test: - PRESSURIZING... , STABILIZE, and TESTING.

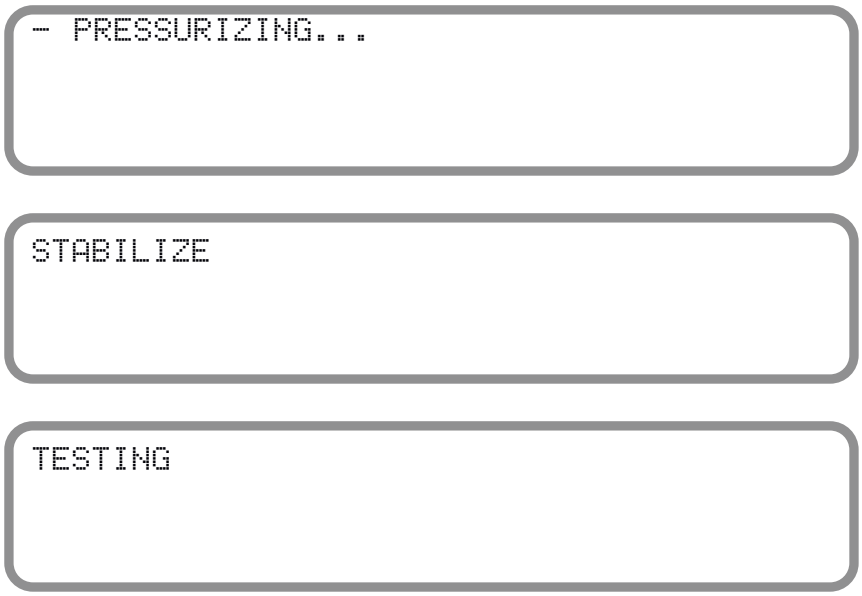


Figure 6-22. Pressurization Test Duty Screens

11. The Pressurization Test automatically finishes within 90 seconds.
    - If there was no leak, the test passes (see top screen of Figure 6-23). Go to step 16.
    - If there was a leak, the test fails (see bottom screen of Figure 6-23). Go to step 12.

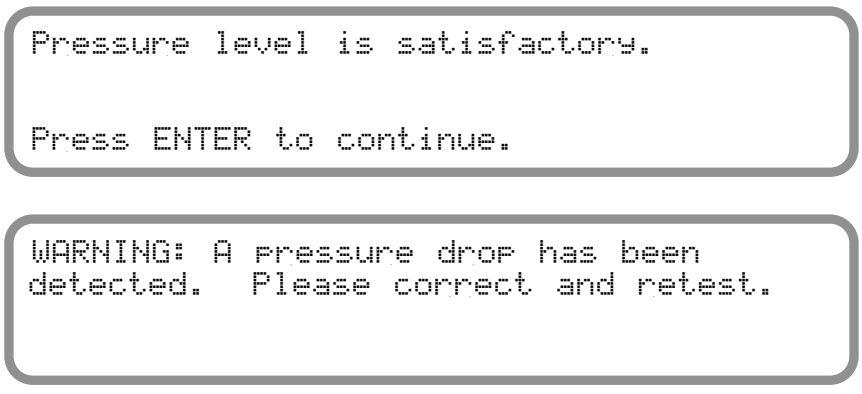

Pressure level is satisfactory.
Press ENTER to continue.
WARNING: A pressure drop has been
detected. Please correct and retest.

Figure 6-23. Pressurization Test Passed Screen and Pressure Test Failed Screen

12. If the probe case assembly Pressurization Test fails, press **ALARM** to silence the audible alarm (and deactivate the external alarm relay).
13. A screen similar to Figure 6-24 appears.

Figure 6-24. Pressuirzation Test Failure Alarm Screen

14. The leak must be located and fixed before proceeding with the run. Otherwise, later on the cultureware will likely leak cell culture medium and become contaminated, which could jeopardize the cultureware's warranty. Potential sources of a leak are:
    a) Tightness of the lower and upper sections of the probe case (don't over tighten them!)
    b) Proper installation of the o-rings
    c) Damage to the o-rings
    d) Tightness of the Luer connections
    e) The instrument itself, if it did not successfully pass the instrument Pressurization Test described earlier in this chapter. Perform this test, if necessary!
15. After each attempt to fix the leak, repeat the Pressurization Test to confirm the leak has been fixed:
    a) If necessary, press **ALARM**
    b) Use ⬅ or ➡ to locate the `Pressure drop detected` alarm
    c) Press **ENTER** to restore the alarm
    d) Press **START** then **SET-UP**
    e) Press **ENTER** if necessary to return to the Active Setup Menu screen (Figure 6-26)
    f) Repeat the probe case assembly Pressurization Test using steps 6 – 11.
16. When the probe case assembly Pressurization Test passes, press **ENTER** to display the following screen.

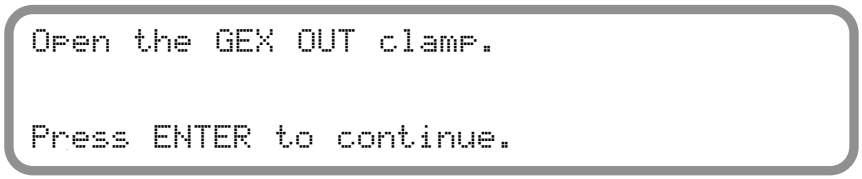


Figure 6-25. Final Pressuirzation Test Screen

17. Ignore the clamp instruction because it is irrelevant when pressure-testing the probe case assembly.
18. Press **ENTER** to return to the Active Setup Menu screen (Figure 6-26).

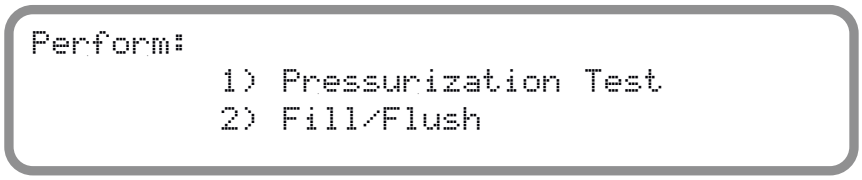


Figure 6-26. Active Setup Menu Screen

19. Disconnect the probe case assembly from the IC and EC gassing lines and connect the other probe case assembly.
20. Repeat steps 6 – 11 to pressure-test the other probe case assembly.
21. When the other probe case assembly Pressurization Test passes, disconnect it from the IC and EC gassing lines.
22. Repeat steps 16 – 19.
23. Reconnect the sterility protectors that were disconnected in step 2.

Sterilization of Probes

The sterilization instructions do not apply to the disposable pH probe.

After the probe case assemblies have passed pressure testing, they can be sterilized. Fill the probe cases with phosphate buffered saline or bicarbonate buffered saline solution until the liquid and electrode are in contact. Be certain the sterility protectors (tubing with white plugs) cover the ends of the probe case tubing. **Loosen the probe cases ¼ turn to allow for thermal expansion in the autoclave.** Be certain the electrical connection at the top of each probe is covered with its screw cap. Put the probe case assemblies in the autoclave so the tubing is facing upward at an angle and is not pinched shut.

Use validated sterility indicators. Run autoclave at 121°C, 55 minutes, slow (liquid cycle) exhaust.

Carefully transfer the sterile probe case assemblies to a laminar flow hood. If liquid has accumulated inside the probe case tubing, allow it to drain into the probe case before attaching the assembly to the flowpath module. Allow the probe case assemblies to cool to room temperature in a laminar flow hood. Re-tighten the probe case bodies by hand **after** they have cooled.

Note: **The sterility protectors contain porous white plugs that cannot survive more than one autoclave cycle. If more than one sterilization cycle must be performed, the sterility protectors must be replaced, or the ends of the tubing must be covered. Contact Account Services for details.**

Cultureware Sterile Assembly

After the probe case assemblies have been sterilized, attach them to the cultureware inside a laminar flow hood. Additionally, in-line media filters, tubing extension lines, and/or sampling septa should be attached at this time, if desired. The following instructions describe the procedures to make these additions to the cultureware. You need to supply sterile gloves, disinfectant in addition to optional sterile in-line filters, tubing extension lines, and any other sterile modifications necessary for a custom configuration. See Appendix B for ordering information on in-line filter assemblies and tubing extension lines.

CAUTION: Do NOT lift the cultureware by its sterile bag! Lift and carry the cultureware by holding it from the underside.

1. To minimize system contamination:
   a) Use aseptic technique.
   b) Inspect the cultureware's sterile bag for holes or tears. Do the same for the optional sampling septa and other sterile accessories that will be used.
   c) Disinfect the outside of the cultureware's bag and the packaging materials of the sterile accessories (if in use) and place them in the sterile hood.

CAUTION: Fully open the bag and remove the cultureware from it while inside the sterile hood. During this step, cultureware tubing can be caught and pulled by the plastic bag! Be very careful to not pull tubing off of their connectors!

   d) Confirm the green tape on the cultureware has brown stripes to indicate sterility conditions by Ethylene oxide.
   e) Remove and discard the foam protectors that are taped to the corners of the cultureware tray. Remove and discard the green striped tape that stretches across most of the tubing.
   f) Carefully place the tubing from the tray onto the surface of the flow hood. Inspect all clamps and sterility covers. Check and finger-tighten all fittings and connections. **Do not over-tighten** clear, plastic components because using too much force can break them!
   g) Confirm the GEX clamp labeled **CLOSE CLAMP BEFORE USE** is open! Now close this clamp. It remains closed for the entire run.
   h) Check that all sterility caps and plugs are in place.

i) Confirm there is no kinked tubing and all clamps are open!

j) Close all clamps on tubing with open ends (sample lines, bottle connections, etc.).

k) Remember to check the tightness of the 3 filters (IC, EC, GEX IN), five Luer plugs on the GEX, and the Luer connection for the in-line filter in the combined Media and F2 line.

If there is any question regarding cultureware sterility, please contact Biovest International Account Services before proceeding.

2. Working in a sterile hood, attach either the autoclave-sterilized, reusable pH probe or the pre-sterilized, disposable pH probe to the cultureware:

   a) Remove the black plastic holder that is taped to the tray and between the two clear chambers. Save this for use with the DO probe case assembly.

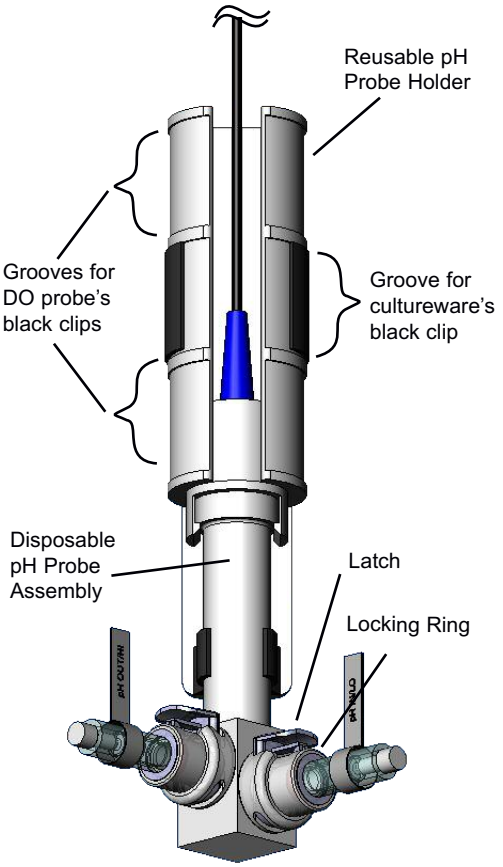
   b) When using the reusable pH probe, remove the autoclaved packaging materials and insert the probe case into the right-hand black clip on the front of the cultureware. The tubing lines connected to the probe case should point to the left and right. Go to step 3.

   c) When using the disposable pH probe, disinfect the:
      - outside of its sterile bag and place it in the laminar flow hood
      - reusable pH probe holder and place it in the laminar flow hood.

   d) Insert the reusable pH probe holder into the right-hand black clip on the front of the cultureware (illustrated at right). Ensure the small black clip on the reusable pH probe holder is facing forward and positioned below the round section held by the cultureware's clip.

   e) Remove and discard the packaging for the disposable pH probe assembly.

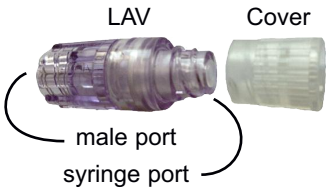
   f) Press the disposable pH probe case into the reusable pH probe holder's black clip and position it as shown at right.



3. Connect the cultureware tubing to the pH probe assembly. These steps apply to both the disposable and reusable pH probe assemblies:
   a) The pH probe assembly replaces the cultureware's tubing adapter between the tubing ends labeled **PH IN/LO** and **PH OUT/HI**. The pH probe case ports are offset from one another—one is higher than the other. Orientation is important!
   b) Rotate the locking rings of the four quick-coupler fittings to the unlocked position. There are two in the pH probe assembly and two in the tubing adapter between the **PH IN/LO** and **PH OUT/HI** labels.
   c) Press and hold the latch of the pH probe's **PH IN/LO** quick-coupler fitting. Remove the fitting from the probe case and discard it.
   d) Press and hold the latch of the cultureware's **PH IN/LO** quick-coupler fitting. Disconnect this fitting from the cultureware's tubing adapter and connect it to the pH probe's exposed port from the previous step. Ensure the latch faces upward.
   e) Release the latch and rotate the locking ring to the locked position.
   f) Repeat the previous two steps for the **PH OUT/HI** connection.
   g) When using the reusable pH probe, save the removed tubing adapter for use at the end of the run.

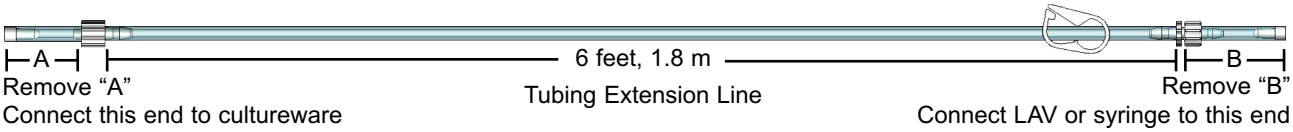
4. Attach the autoclave-sterilized DO probe case assembly to the cultureware:
   a) Attach the two black plastic clips that were set aside as the first task of step 2 above to the correct locations depending on which type of pH probe is in use:
      - when using the reusable pH probe, press the two black clips on to the pH probe case. Locate both black clips below the clip of the cultureware that is holding the pH probe case itself.
      - when using the disposable pH probe holder, press the two black clips on the two grooves of the reusable pH probe holder.
   b) Similar to the pH probe case, the DO probe case ports are offset from one another—one is higher than the other. The difference is more subtle than for the pH probe case.Orientation is important! The liquid inlet port is lower than the liquid outlet port.
   c) Locate in the cultureware the two red Luer-Lok fittings connected together by a polypropylene double female Luer fitting. They are near the bottom of the GEX. Two clear Tygon tubing lines connect to these red Luers: **DO IN** tubing line and the unlabeled DO OUT tubing.
   d) The DO probe case inlet tubing line connects to the culturewares **DO IN** tubing line.
   e) The DO probe case outlet tubing line connects to the culturewares **DO IN** tubing line.
   f) Disinfect the white Luer-Lok fittings of both DO probe case tubing lines.
   g) Rotate the DO probe case in the black ckips so the DO probe case inlet tubing line reaches the **DO IN** tubing line.
   h) Loosen white Luer-Lok (on DO inlet tubing line). Loosen polypropylene fitting connected to red Luer-Lok (on the **DO IN** tubing line).
   i) Remove and discard the white Luer-Lok, short tubing, and white plug.
   j) Disconnect **DO IN** red Luer-Lok (from polypropylene fitting) and connect it to exposed female Luer of DO probe case inlet tubing line.
   k) Rotate DO probe case in the black ckips so DO probe case outlet tubing line reaches the unlabeled DO OUT tubing line.
   l) Repeat steps h–j to connect DO Out tubing line's female Luer to remaining red Luer-Lok (of unlabeled DO OUT tubing line).
   m) Save the removed double female Luer fitting for use at the end of the run.

5. Optional, sterile items should be added to the cultureware while the cultureware is still in the sterile laminar cabinet. Examples of these options are explained below. Appendix B has ordering information for these items. Use LAVs or tubing extension lines together or separately, depending on your application and facility.

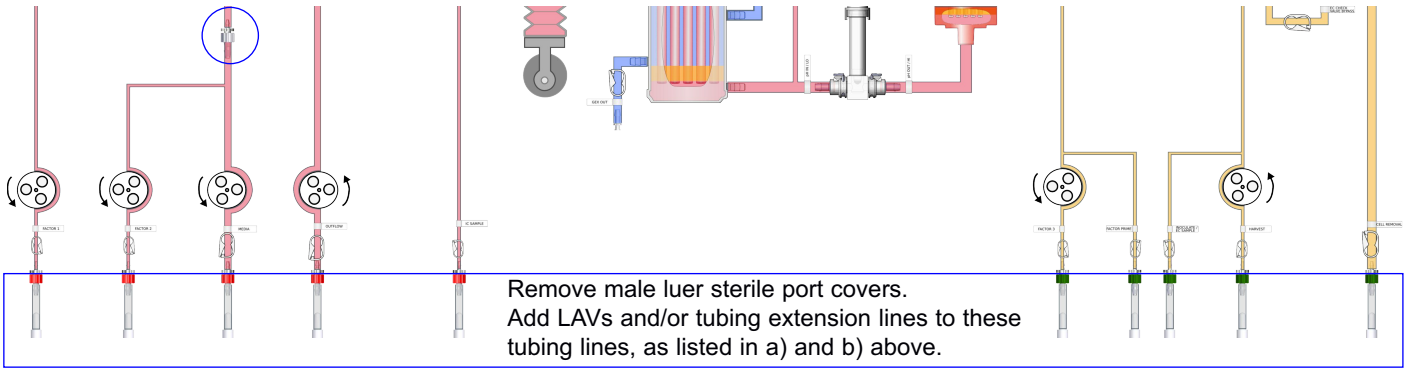


a) Luer Activated Valves (LAV) allow you to connect and disconnect syringes aseptically outside of a sterile laminar cabinet. Usage instructions are provided with the package of LAVs.
Use LAVs on cultureware's four syringe ports:
IC SAMPLE, **FACTOR PRIME**, **INOCULATE/EC SAMPLE**, **CELL REMOVAL**

b) Tubing extension lines provide longer tubing lengths to allow you to aseptically connect bags, bottles, and syringes in a nearby sterile laminar cabinet. Use them to extend cultureware's ten tubing lines:
• **MEDIA**, **OUTFLOW**, **CELL REMOVAL** (use 600101-072)
• **F1**, **F2**, **F3**, **HARVEST**, **IC SAMPLE**, **FACTOR PRIME**, **INOC/EC SAMPLE** (use 600100-072).



c) In-line media filters minimize the risk of a contaminant entering the flowpath and typically are used for **MEDIA** and **F2** because these deliver nutrient fluids into the flowpath. Use the in-line filter listed in Appendix B (not shown) at the blue circled location, or add your own filter assembly at the end of an individual tubing line.


Remove male luer sterile port covers.
Add LAVs and/or tubing extension lines to these tubing lines, as listed in a) and b) above.

6. The flowpath is now completely assembled and ready to be installed in the *MAXIMIZER* incubator.

Cultureware Installation

To install the cultureware, you will need: 3 Tygon gassing lines (red, green, and blue Luers) , pH probe cable, DO probe cable, and temperature probe that were supplied with the *MAXIMIZER.* The black-collared Tygon gassing line for off-gassing may be needed (see **Pre-inoculation Procedures**).

1. Connect pH and DO cables to their respective probes. Save the electrical caps for future autoclave cycles and for probe storage.
2. Connect the temperature probe to the non-invasive, in-line fitting in the large-diameter tubing near the middle of the BRX(s).
   a) Note the fitting has two small plastic knobs.
   b) Note the temperature probe's connector has two curved channels.
   c) Align the channels' openings with the small plastic knobs.
   d) While pushing the tip of the probe into the fitting, rotate the temperature probe connector ¼-turn to lock the probe in the fitting.
   e) Push the temperature probe's insulation over the fitting.
3. Set aside the two metal screws and the white plastic threaded cap for the circulation pump. **Do not skip this step.** It can prevent cultureware damage!
4. Carefully place the cultureware into the incubator. The upper tray sits **behind** the metal ciculation pump bracket. Do not pinch any tubing between the tray and incubator. Allow the long tubing lines to hang in front of the instrument. Do **NOT** load any pump heads at this time!
5. Connect pH, DO, temperature, and EC ultrasonics cables to their respective bulkhead connections. Connect Tygon gassing lines to their color-coded bulkhead fittings, then attach them to the IC, EC, and (GEX) IN filters.
6. Simultaneously slide the upper and lower portions of the cultureware's bellows pump onto the metal bracket and crank pin. *If necessary*, grasp the black, hexagonal part of the bellows pump while rotating the black, flat arm of this pump to align it with the crank pin. Do not loosen the bellows pump assembly!
7. Fully thread the two metal screws until their heads touch the bracket.
8. Thread the white plastic threaded cap
9. Press the tubing lines into channels at the edge of the incubator's opening.
10. The cultureware is now ready for the Pressurization Test.

Cultureware Labels

Certain cultureware tubing lines and connections are labeled with unique names to differentiate them and make their usage clear. These labels are referred to throughout the following procedures and in the LCD's onscreen instructions.

Because the LCD's space is limited, some of its label names are shortened from what is printed on the labels that are on the cultureware. The following table relates the actual names to the names shown on the LCD. Use this information if there is any uncertainty about which label is being referred to by the onscreen instructions. Not all cultureware labels are used in the onscreen instructions.

Label names in this manual primarily use the full cultureware label names.

Cultureware Label Name	LCD Label Name
CELL REMOVAL	CELL REMOVAL
EC CHECK VALVE BYPASS	EC Check Valve Bypass
EC FLUSH IN	EC FLUSH IN
EC RETURN	EC RETURN
FACTOR #1	F1
FACTOR #2	F2
FACTOR #3	F3
FACTOR PRIME	FACTOR PRIME and EC FACTOR PRIME
GEX OUT	GEX OUT
HARVEST	HARVEST
IC	IC Vent
IC RETURN	IC RETURN
IC SAMPLE	IC SAMPLE
INOCULATE/EC SAMPLE	INOC/SAMPLE
MEDIA	MEDIA
OUTFLOW	OUTFLOW
POST BRX DO	POST BRX DO
PRE BRX DO	PRE BRX DO
EC	not displayed onscreen
DO IN LO	not displayed onscreen
GEX IN	not displayed onscreen
PH IN/LO	not displayed onscreen
PH OUT/HI	not displayed onscreen
CLOSE CLAMP BEFORE USE	not displayed onscreen

Cultureware Pressurization Test

After the cultureware has been installed in the *MAXIMIZER* incubator, it must be tested for leaks. This procedure can be performed with the incubator door open or closed.

1. Press **START** then **SET-UP**.

Figure 6-27. Active Setup Menu Screen

2. Press **1** to select Pressurization Test.

Figure 6-28. Begin Pressurization Test Screen

3. Press **ENTER** to begin the test.

4. Open or close the clamps as instructed on the next five screens (shown below) to prepare the cultureware for the Pressurization Test. Make certain that closed clamps are centered over the tubing and completely pinch it. Press **ENTER** when instructed.

```
Close OUTFLOW line clamp.
Close MEDIA line clamp.
Close F1, F2 and F3 line clamps.
Press ENTER to continue.
```

```
Close HARVEST and INOC/SAMPLE
line clamps.

Press ENTER to continue.
```

```
Close IC SAMPLE clamp.
Open PRE and POST BRX DO clamps.

Press ENTER to continue.
```

```
Open IC Vent and IC RETURN clamps.
Close EC FLUSH IN clamp.
Close FACTOR PRIME clamp.
Press ENTER to continue.
```

```
Close CELL REMOVAL line clamp.
Open EC RETURN clamp.
Close GEX OUT clamp.
Press ENTER to continue.
```

Figure 6-29. Instructions Screens 1-5 for Pressurization Test

5. The LCD updates to show the three automated stages of the Pressurization Test: - PRESSURIZING... , STABILIZE, and TESTING.

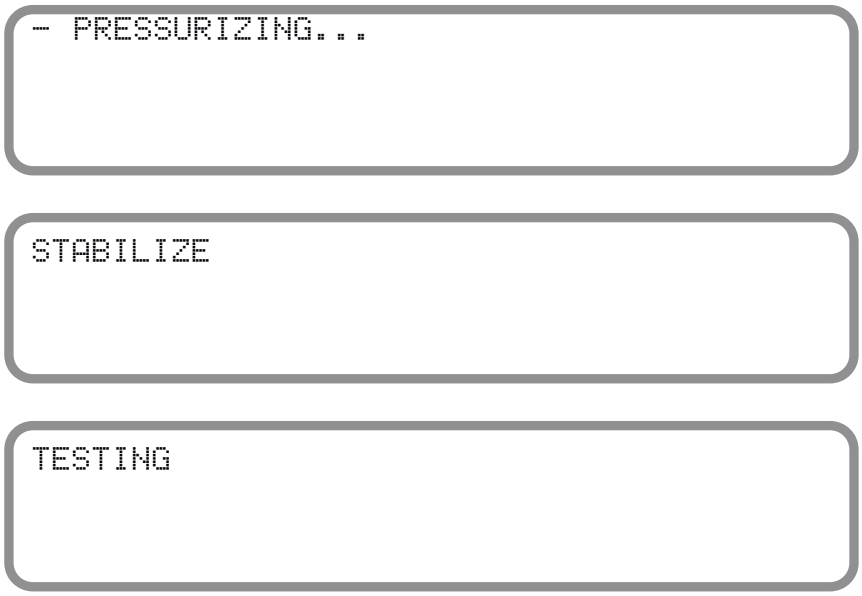


Figure 6-30. Pressurization Duty Screens

6. The Pressurization Test automatically finishes within 90 seconds.
   - If there was no leak, the test passes (see top screen of Figure 6-31). Go to step 12.
   - If there was a leak, the test fails (see bottom screen of Figure 6-31). Go to step 7.

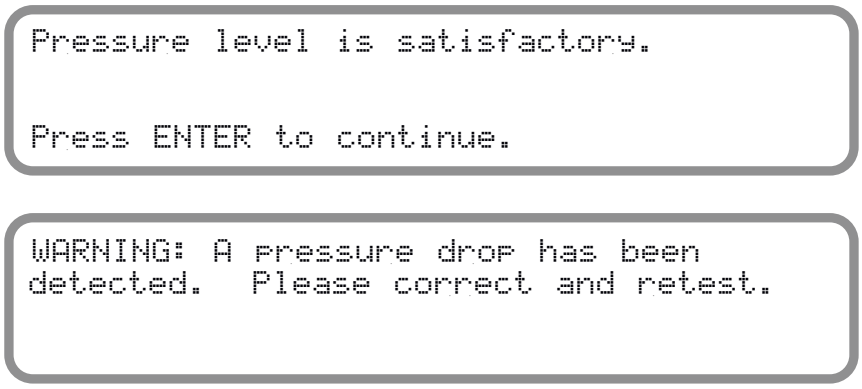

Pressure level is satisfactory.
Press ENTER to continue.
WARNING: A pressure drop has been
detected. Please correct and retest.

Figure 6-31: Pressurization Test Passed Screen and Pressure Test Failed Screen

7. If the cultureware Pressurization Test fails, press **ALARM** to silence the audible alarm (and deactivate the external alarm relay).
8. A screen similar to Figure 6-16 or 6-17 appears.
   - If EC leaked, the second number is > 0. For example: 2 in Fig. 6-32.
   - If IC leaked, the third number is > 0. For example: 2 in Fig. 6-33.

Figure 6-32. Example Pressuirzation Test Alarm Screen: EC Leak

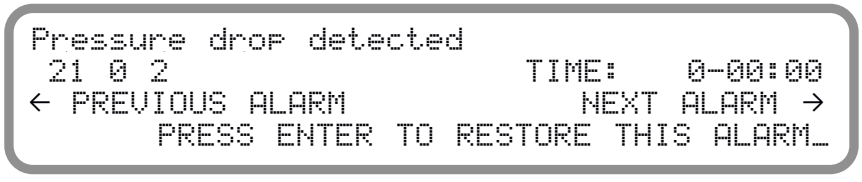


Figure 6-33. Example Pressuirzation Test Alarm Screen: IC Leak

9. The leak must be located and fixed before proceeding with the run. Otherwise, later on the cultureware will likely leak cell culture medium and become contaminated, which could jeopardize the cultureware's warranty. Focus your efforts on finding and fixing the leak in either the IC circuit or EC, as indicated in the `Pressure drop detected` alarm screen.
10. Potential sources of a leak are:
    a) Clamp was opened that should have been closed
    b) Tubing is not centered in a closed clamp, particularly F1, F2, F3, Harvest, Factor Prime, Inoc/EC Sample
    c) Lower and upper sections of the probe cases weren't tightened (don't over tighten them!)
    d) Tab of pH IN/LO connector or pH OUT/HI connector was not fully pressed in while connecting it on to the pH probe case port fitting, which damaged the o-ring or pushed it out-of-place
    e) Loose male/female Luer connection
    f) Loose male Luer plug
    g) Cable ties were installed on tubing that connect to DO or pH probe case port fittings
    h) The instrument itself, if it did not successfully pass the instrument Pressurization Test described earlier in this chapter. Perform this test, if necessary!
11. After each attempt to fix the leak, repeat the Pressurization Test:
    a) If necessary, press **ALARM**
    b) Use ⬅ or ➡ to locate the `Pressure drop detected` alarm
    c) Press **ENTER** to restore the alarm
    d) Press **START** then **SET-UP**
    e) Press **ENTER** if necessary to return to the Active Setup Menu screen (Figure 6-27)
    f) Repeat the instrument Pressurization Test using steps 2 – 6.

Note: **If you cannot find the leak after a few attempts using reasonable efforts, contact Biovest's Account Services group before proceeding! Keep the cultureware intact and sterile to not jeopardize the product warranty or incur re-sterilization expense and delay!**

12. When the cultureware Pressurization Test passes, press **ENTER** to display the following screen.

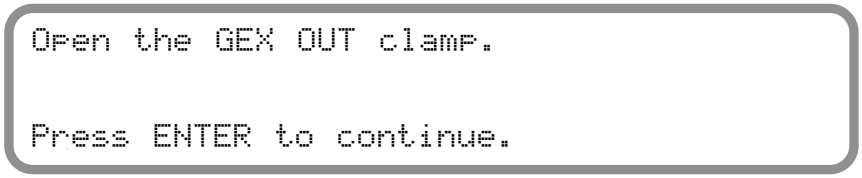


Figure 6-34. Final Pressuirzation Test Screen

13. Open the **GEX OUT** clamp!
14. Press **ENTER** to return to the Active Setup Menu screen (Figure 6-27).
15. After the cultureware passes the pressurization test, proceed with the **Fill / Flush** procedure.

CAUTION: Do not proceed to Fill / Flush unless the culureware passes the Pressurization Test!

Fill/Flush Procedure

After the flowpath has been installed and tested for leaks, the cultureware can be primed with sterile medium.

Notes:

1. **This procedure requires sterile medium and a waste (collection) bottle large enough to contain this medium. A single BRX cultureware (600058-xxx) should be flushed by collecting at least 6 L. A double BRX cultureware (600068-xxx) should be flushed by collecting at least 8 L. When this procedure is completed, you will also need sterile medium and factor solutions as well as one sterile waste bottle to begin the Pre-inoculation phase of cultureware setup.**

2. **This procedure operates only with the medium pump head loaded. Do NOT load any other pump heads until the procedure is completed and the onscreen instructions tell you to do so.**

1. Press **START** then **SET-UP**, if necessary, to display the following screen.

Figure 6-35. Screen to Begin Fill/Flush Procedure

2. Press **2** to begin the Fill & Flush procedure.

3. Open or close the clamps as instructed on the next four screens (shown below) to prepare the cultureware for Fill/Flush. Make certain that closed clamps are centered over the tubing and completely pinch it. Even if you have recently finished the cultureware pressurization test, make certain these clamps are adjusted properly. Press **ENTER** when instructed.

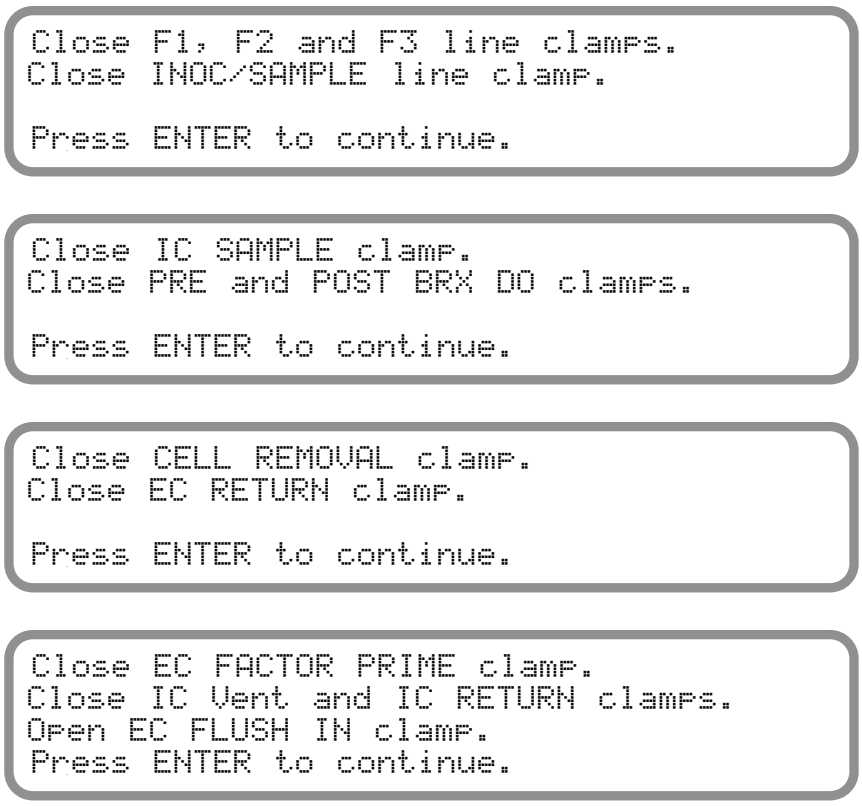

Close F1, F2 and F3 line clamps.
Close INOC/SAMPLE line clamp.
Press ENTER to continue.
Close IC SAMPLE clamp.
Close PRE and POST BRX DO clamps.
Press ENTER to continue.
Close CELL REMOVAL clamp.
Close EC RETURN clamp.
Press ENTER to continue.
Close EC FACTOR PRIME clamp.
Close IC Vent and IC RETURN clamps.
Open EC FLUSH IN clamp.
Press ENTER to continue.

Figure 6-36. Instructions Screens 1-4 for Fill/Flush

4. The fifth screen tells you to load the medium pump head. Follow the instructions in Appendix E to do this step. Attach the loaded pump head to the mounting position labeled "MEDIA". When this is done, aseptically attach the medium pump tubing to the bottle (or bag) of medium that will be used to flush the flowpath. Then, close the Outflow clamp.

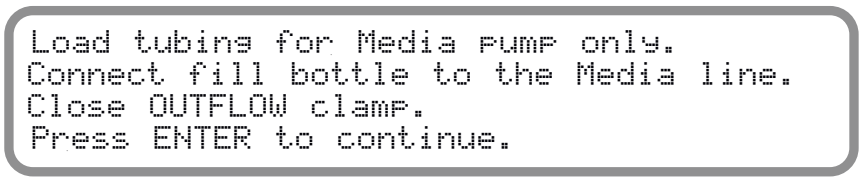


Figure 6-37. Instructions Screen 5 for Fill/Flush

Note: **The Media and Outflow pump tubing segments must be loaded into size 7016 peristaltic pump heads. Factor and Harvest pump tubing segments must be loaded into size 7014 peristaltic pump heads.**

5. The sixth screen tells you to attach the Harvest line aseptically to the collection bottle. This bottle should be large enough to contain the medium intended for flushing. When this is done, disconnect the IC tubing line from its bulkhead connection. This step acts as a safety measure against medium leaking through the vent filter and flowing inside the instrument.

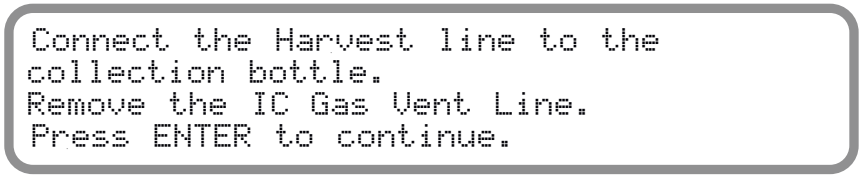


Figure 6-38. Instructions Screen 6 for Fill/Flush

6. The next screen tells you to close the two EC Check Valve Bypass clamps. After that, open the Medium and Harvest line clamps for the inlet and outlet lines during the Fill/Flush procedure.

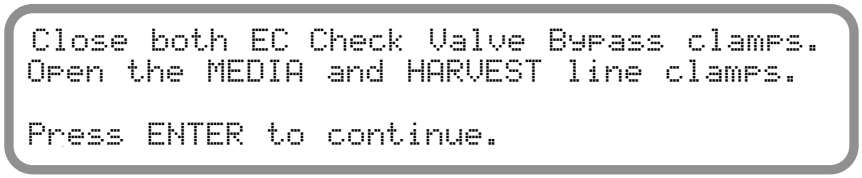


Figure 6-39. Instructions Screen 7 for Fill/Flush

7. The next screen tells you to inspect the flowpath before the Medium pump is started. Carefully follow the tubing route from the Medium bottle to the Harvest bottle. When the route meets another piece of tubing, be certain that the appropriate clamps are shut to force medium to flow in only one direction. Verify that closed clamps are centered over their tubing and **completely** occlude the tubing. Be certain that tubing is not pinched between the cultureware tray and the incubator walls.

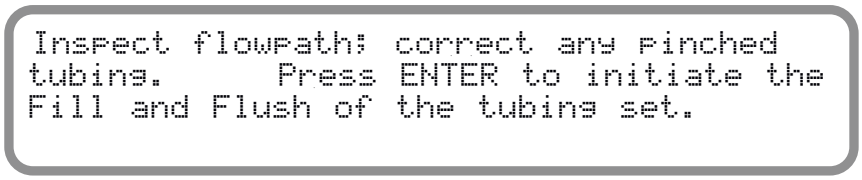


Figure 6-40. Instructions Screen 8 for Fill/Flush

8. The correct route of Fill/Flush is described below and shown in Fig. 6-41:
   - Medium bottle to Medium pump (MP)
   - MP to top of IC chamber
   - Bottom of IC chamber to Circulation Pump (CP)
   - CP to GEX to pH probe
   - pH probe to bottom (IC inlet) of BRX(s)
   - Top (IC outlet) of BRX to POST-BRX sample line
   - Sample line to EC FLUSH IN line
   - EC FLUSH IN to bottom (ECS inlet) of BRX(s)
   - Top (EC outlet) of BRX(s) to Harvest line
   - Harvest line to Waste (collection) bottle

Note: **Flush medium may flow from the BRX IC to the BRX EC directly through the fiber wall, rather than via the EC Flush In line, which is acceptable.**

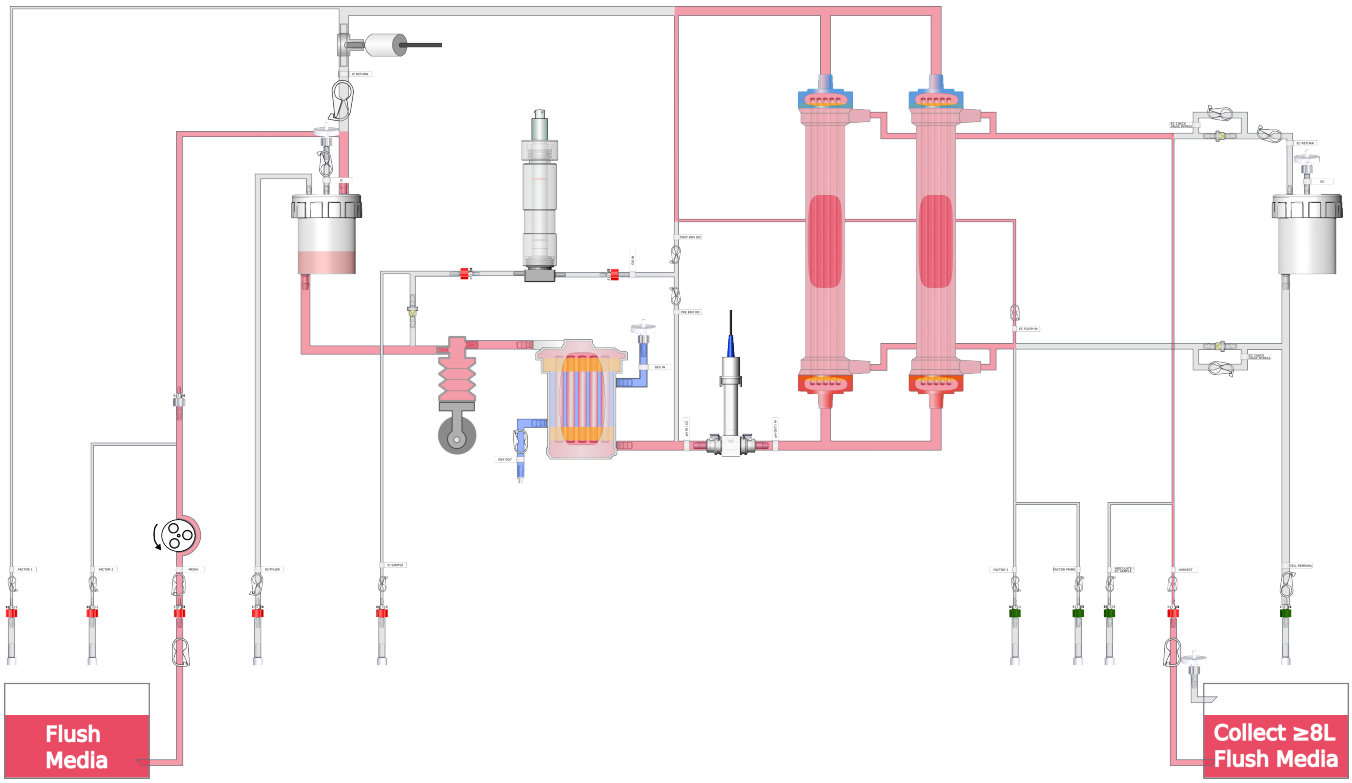


Figure 6-41. Route of media flow during initial Flush

9. When you are sure the Fill/Flush route is free from obstructions in the previous step, press **ENTER**.

10. The screen shown in Figure 6-42 appears. Also, the Medium pump automatically turns on and runs at 4320 ml/hr to flush the wetting agent out of the hollow fibers in the BRX(s).

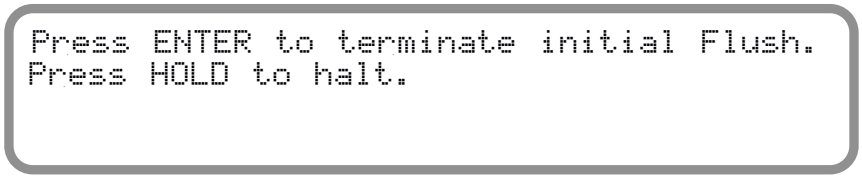


Figure 6-42. Duty Screen 1 for Fill/Flush

11. `Press ENTER to terminate initial Flush.` means: press **ENTER** to stop the MP after the trouble-free collection of at least 8 L (2 BRXs) or 6L (1 BRX) of flush medium into the container connected to the Harvest tubing line. This step requires about 1.5 – 2 hours (using default MP speed).

12. `Press HOLD to halt.` means:
    a) Press **HOLD** when one of the following occurs:
       - Flow route of medium is not according to the schematic (Fig. 6-41)
       - Cell culure media leaks from the cultureware
       - One of the following three *Notes* (below) is a problem
    b) Correct the problem, then press **1** to `Continue current activity` (screen not shown):

Notes:

1. **If the route of medium flow is different from what is illustrated or medium leaks from the cultureware, press HOLD to stop the procedure temporarily. After the problem has been resolved, resume Fill/Flush procedure by selecting pressing 1 to `Continue current activity`.**
2. **During this phase of the Fill/Flush procedure, it is CRITICAL that the level of medium in the IC chamber does NOT continue to rise and fill the IC chamber. If the medium continues to rise beyond ~5 cm (2″) of medium, ensure that the IC vent clamp is completely closed. If the problem continues, there may be a blockage downstream of the IC Chamber. If you cannot resolve this, press the HOLD key and contact Biovest!**
3. **During this phase of the Fill/Flush procedure, the EC chamber receives NO Fill/Flush medium and should remain DRY. If Fill/Flush medium is**

entering the EC chamber, completely close the EC Return clamp and the lower Check Valve Bypass clamp. If the problem persists, contact Biovest!

13. The first phase of Fill/Flush is complete.
14. The results of the instructions in the screen (Figure 6-43):
    a) Opening the **EC RETURN** clamp and closing the **HARVEST** clamp diverts the flow of cell culture medium from exiting the Harvest line to entering the Expansion Chamber. (This is the first time the Expansion Chamber should become wet.)
    b) Pressing **ENTER** causes the Medium pump to automatically turn on and run at 4320 ml/hr.

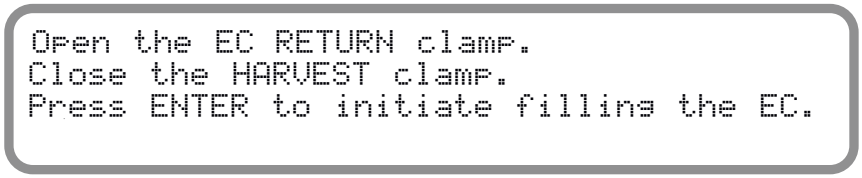


Figure 6-43. Instructions Screen 9 for Fill/Flush

15. The meaning of the instructions in the following screen (Figure 6-44) is: Press **ENTER** when you see that the Expansion Chamber is approximately half-full. This stops the Media Pump (and changes the screen). This step only takes about 1.5 – 2 minutes.

CAUTION: You must decide when to stop the pump! It will NOT automatically stop! Do NOT become distracted during this brief step! Otherwise the Expansion Chamber could flood and leak cell culture medium—possibly ruining the cultureware and damaging the instrument!

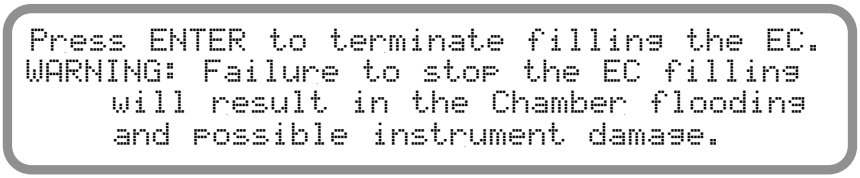

Press ENTER to terminate filling the EC.
WARNING: Failure to stop the EC filling
will result in the Chamber flooding
and possible instrument damage.

Figure 6-44. Duty Screen 2 for Fill/Flush

16. Open the **POST-BRX DO** clamp (to prime the DO circuit during the pre-inoculation period). Be certain the **EC FLUSH IN** clamp is centered over its tubing, close the clamp, and ensure the tubing is completely pinched shut. Wrap the **EC FLUSH IN** clamp with tape to keep it securely closed. This clamp remains shut for the remainder of the run.

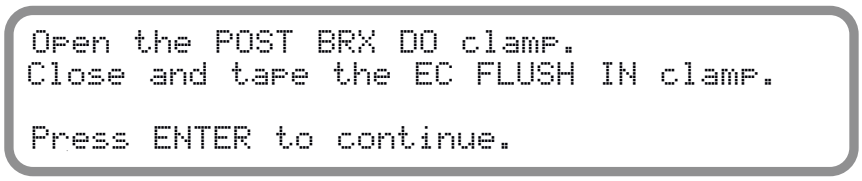


Figure 6-45. Instructions Screen 10 for Fill/Flush

17. Re-attach the reusable Tygon IC tubing to the **IC** bulkhead connector. Open the **IC** Vent (see note below) and **IC RETURN** clamps. Be certain the **IC RETURN** clamp does not pinch the circulation tubing after it has been opened.

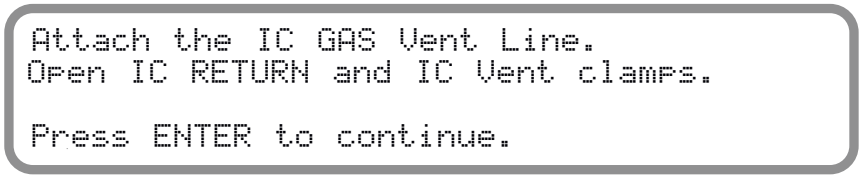


Figure 6-46. Instructions Screen 11 for Fill/Flush

Note: **The IC Vent clamp's label reads IC, not IC VENT.**

18. The next screen tells you to load the remaining five pump heads with their respective tubing segments. **Remember that the Outflow and Harvest pumps are loaded differently from the other pumps because they remove media from the flowpath!**
    a) Aseptically attach a waste bottle to the Outflow pump.
    b) Asepically attach a fresh medium bottle (or bag) to the Media pump, if necessary.
    c) Aseptically attach Factor bottles to their respective tubing lines.
    d) Aseptically replace the Flush Collection container on the Harvest line with an empty, sterile bottle.
    e) Open the **OUTFLOW** clamp.
    f) Discard the collected flush medium.

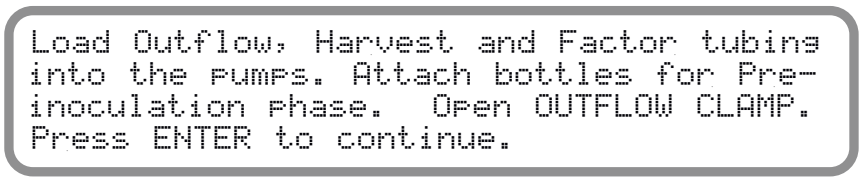

Load Outflow, Harvest and Factor tubing
into the pumps. Attach bottles for Pre-
inoculation phase. Open OUTFLOW CLAMP.
Press ENTER to continue.

Figure 6-47. Instructions Screen 12 for Fill/Flush

Note: **Using Outflow and Media pumps is necessary at this point. You must decide when/if Factor and Harvest pumps are needed. If so, and they are attached to their respective bottles, open their clamps now to allow their pumping later on.**

19. The next screen informs you that Fill/Flush is finished. Pressing enter causes the next screen to appear (Figure 6-49).

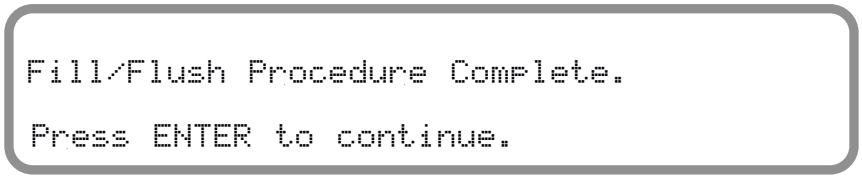


Figure 6-48. Instructions Screen 13 for Fill/Flush

20. The instructions in the next screen (Figure 6-49) mean that you need to temporarily leave this Fill/Flush screen to go into the Process Control screens to:
    - Change the default pH setpoint to what is best for your particular cell line. This ensures the Pre-inoculation phase to follow adjusts the cell culture medium to what is necessary for the pending inoculum.
    - Start EC Cycling by changing the default `Delay` from `7-00:00` to `0-00:00`. Starting EC Cycling speeds pH adjustment throughout the cultureware. The remaining EC Cycling defaults are OK: `70`, `15`, `15`.

    a) Press **PROCESS CONTROL**

    b) Press **ENTER** until `pH Setpoint` appears

    c) Enter the pH that corresponds to mid-log phase growth for the pending inoculum, if known. Otherwise, 7.1 – 7.3 often works for many cell lines.

    d) Press **ENTER** until `EC Cycling` appears

    e) Change `DELAY` time to `0-00:00`

    - Suggest a Circulation Rate of 250 ml/min for 1 BRX or 2 BRX cultureware during first few hours of pending Pre-Inoculation Phase.

CAUTION: Do NOT exceed 500 ml/min Circulation Rate when using cultureware with 1 BRX at any time!

```
Set PROCESS CONTROL parameters for the
Pre-inoculation phase.
Remember: Begin cycling and set pH.
Press ENTER to continue.
```

Figure 6-49: Instructions Screen 1 for Pre-inoculation Phase

Notes: In addition to entering the desired pH setpoint, calibrate pH approximately 30 minutes after starting the Pre-inoculation phase (step 24 below) when the on-line pH will have reasonably equilibrated. This ensures accurate pH control. Calibration is described below in Maintenance Procedures.

21. If ACULINK-DATA LOG or a printer is connected, enable them and enter the desired Logging Interval by pressing:

    a) **SET-UP**

    b) **ENTER**, if necessary, to display `Logging Interval`

    c) Number keys for the desired logging frequency

    d) **ENTER**

21. When the changes to Process Control and Logging Interval are complete, press **START** then **SET-UP** to return to the screen shown in Figure 6-49.
22. Press **ENTER** to advance to the next screen, which is provided as a safety feature. If you have performed the Fill/Flush procedure with the incubator door open, close the door to activate the incubator heater.

Figure 6-50. Instructions Screen 2 for Pre-inoculation Phase

23. Press **ENTER** to proceed to the last screen in the Fill/Flush procedure (Figure 6-51).
24. Press **ENTER** to display the Monitor screen. The following actions have automatically occurred:
    - Elapsed time has reset to `0-00:00`
    - `SETUP` has ended and `RUNNING` is the active mode of operation
    - Active Process Control is active (Active Process Control LED is on), so logging, alarms, setpoint control and other activities are now active
    - See Note on next page

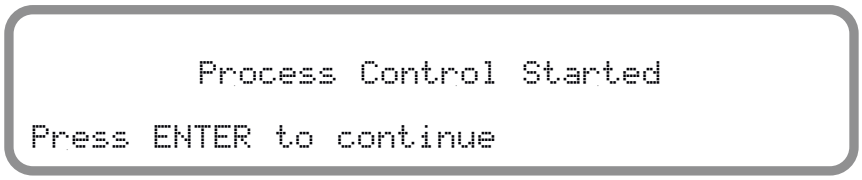


Figure 6-51. Instructions Screen 3 for Pre-inoculation Phase

25. Press **ALARM** to silence the audible alarm and deactivate the external alarm relay. Alarms normally occur (such as temperature, pH and DO) immediately when mode `RUNNING` mode begins. Review the alarms stored in the queue to ensure unusual alarms are not present.
26. Press **MONITOR** and watch temperature, pH and DO readings. After approximately 20 minutes they will be equilibrated and likely within the user-defined `LOW` and `HIGH` alarm limits as defined in Process Control.
27. Press **ALARM**. Individually restore each stored alarm by using arrow keys to locate each parameter that is in its alarm range and pressing **ENTER** to restore its alarm. Press **MONITOR**.

28. If pH does not reach the entered setpoint and equilibrate within its alarm range in order for the pH alarm to be restored, it is likely that pH must be calibrated, which should be done regardless to ensure pH is properly controlled. After RUNNING mode's elapsed time has reached approximately thirty minutes (0-00:30), on-line pH likely will have reasonably equilibrated, so calibrate the pH probe.

Note: **The volume of media within the flowpath — and particularly the Integration Chamber — may be very low when Active Process Control starts. This is normal! This may result in air bubbles circulating through the IC circuit, which is undesirable but does not cause damage. If this occurs, use the Pumps Override Control to set the Circulation Pump rate to 0 ml/min and the Media Pump rate to 800 ml/hr. When the Outflow pump begins drawing medium out of the top of the Integration Chamber, clear the Circulation Pump and Media Pump overrides.**

MAINTENANCE PROCEDURES

This part of **Operating Procedures** describes various procedures that are used during the pre-inoculation and post-inoculation phases of a culture. The intent is to present them only once, as a group, so as to be easier to locate in the future.

Sampling

The procedure on the following page is used to remove samples of medium from the flowpath. These samples can be from either fluid circuit:

- IC for metabolite, pH, or DO analysis or probe calibration
- EC for various product analyses

Details specific to calibrating the pH and DO probes are provided in the next section, **Probe Calibration**.

Post-BRX IC samples may be used to calculate metabolic uptake rates and production rates. (See Appendix D for the formulae in these calculations.) Pre-BRX IC samples may be used to calibrate the pH probe. Post-BRX IC samples may be used to calibrate the DO probe.

Samples from the EC port are used for offline analysis of secreted product. EC sample analysis is affected by EC chamber volume. Remove EC samples at consistent EC chamber volumes to simplify data analysis.

Recommended schedules of sampling for metabolite and product analysis, as well as for probe calibration, are provided in **Post-inoculation Procedures**.

Sampling involves the removal of stagnant medium in the sample port, followed by removal of fresh flowpath medium for offline analysis. The stagnant medium is called a **pre-sample** and is to be discarded. The pre-sample volume from the IC and EC sample ports should be 3 ml. Pre-samples **must** be removed and discarded before the analysis sample is withdrawn. This is true even if a post-BRX sample immediately follows a pre-BRX sample (or vice versa).

Use aseptic technique to perform all sampling operations according to the following protocol:

1. Prepare sterile syringes, disinfectant, sterile gauze, and sampling tubes. (If septa are attached to the sampling ports, use 21 gauge needles on the syringes as described below.)
2. Disinfect the sampling port. If you use caps, wipe the junction of the cap and sample port fitting. If you use luer-activated valves, remove the dust cover and wipe the surface of the LAV with an approved disinfectant (see usage instructions that came with the LAVs). If you use septa, wipe only the surface that will be punctured by the needle.
3. Attach the pre-sample syringe to the sample port. If you use caps, remove the cap and protect it by covering it with sterile gauze. If you use LAVs, simply connect the syringe to the LAV, which opens inside of the LAV. If you use septa, remove the needle guard, then puncture the septum until the tip of the needle can be seen inside the sample port.
4. Adjust the **PRE-BRX** and **POST-BRX** clamps to permit the desired IC sample. Open the clamp to the sample port. Remove the pre-sample volume (3 ml for IC or EC samples).
5. Close the sample clamp. Replace the pre-sample syringe with the sample syringe (for offline analysis). If you use LAVs, wipe the LAV with an approved disinfectant after disconnecting the pre-sample syringe and again before connecting the sample syringe It's unnecessary to reconnect the dust cover between the pre-sample and sample syringes. If you use septa, you can detach the syringe from the needle and leave the needle in the septum while only the syringe is replaced.
6. Open the sample clamp again. Remove the analysis sample.
7. Close the sample clamp. Remove the sample syringe. Replace the sample syringe with a sterile syringe. This syringe may be used for the pre-sample during your next sampling. If you use caps on sample ports, replace it now, and disinfect the junction of the cap and sample port. If you use LAVs, do not leave a syringe connected to the LAV. Wipe it as previously mentioned and reconnect the dust cover. If you used a septum, remove the syringe plus needle, and wipe any drops of medium from the septum's surface.
8. Seal the analysis syringe with its cap or the needle guard.
9. While performing the above tasks, perform the software steps needed for calibration (see **Probe Calibration** procedure below).

10. If this sample is used for pH or DO calibration, proceed immediately to the offline pH meter or blood-gas analyzer (or equivalent), and obtain the desired value. Then, return to the *MAXIMIZER* and complete the software steps in the calibration sequence. If this sample is used for other metabolites, store it appropriately before analysis.

Note: **To avoid inaccurate readings due to changes in pH or DO inside the syringe, you should prepare the offline meter or analyzer in advance of removing the sample, and measure pH or DO immediately after the sample has been withdrawn. Samples that are used for other metabolites or for product analysis should be stored appropriately to avoid degradation before the desired analyte can be measured.**

Probe Calibration

For pH probe calibrations, use samples from the pre-BRX sample line; for DO probe calibrations, use samples from the post-BRX sample line. Calibrate probes according to the following schedule: pH probe, 2 – 3 times per week; DO probe, 1 – 2 times per week.

The instructions below are specific to calibrating either the pH or DO probe. These instructions do not cover details of aseptic connection, removing samples from the flowpath, opening and closing clamps, etc. that were covered in the preceding section, ***Sampling***.

CAUTION: Calibrate in advance the offline pH meter or blood gas analyzer (or equivalent) as recommended by the manufacturer! If this is not done, the use of this equipment to calibrate the MAXIMIZER pH or DO probe can result in faulty pH control or DO readings.

1. Check **PRE-BRX** and **POST-BRX** clamps to verify that the desired clamp is open (pre for pH, post for DO). Only one of these clamps should be open at one time. If the desired IC sample can be taken with the clamp that happens to be open, the pre-sample can be taken immediately. Any time the open clamp changes, wait approximately 2 minutes before proceeding to step 2 to allow the medium in the common tubing line to change. This can be monitored by changes to the DO reading on the Monitor screen.
2. Press **CALIBRATE** to display the following screen. This screen appears only for the first calibration for each run. After the first calibration is completed, pressing **CALIBRATE** displays the screen in Figure 6-56.

Figure 6-52. Main Menu Probe Calibration Screen before Sampling

3. Remove and discard the pre-sample volume from the cultureware.
4. Replace the pre-sample syringe with the analysis sample syringe.

5. Press **1** (Take Sample) to display the following screen.

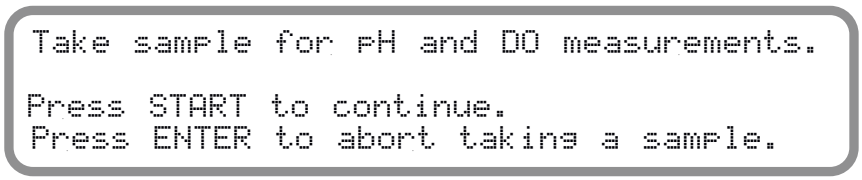


Figure 6-53. Probe Calibration Screen 2

6. To abort the calibration either before or after removing the sample:
   a) Press **ENTER**
   b) The screen in Figure 6-52 re-appears (to re-start calibration, if desired)
   c) Press **MONITOR** to leave the calibration screen (and discontinue calibration)
7. To continue with the calibration:
   a) Remove the analysis sample
   b) Immediately press **START** (to store the current probe readings and advance the screen, Figure 6-45)
   c) The elapsed time for sample taken at is when **START** was pressed. This also is logged by the printer or ACULINK-DATA LOG, if in use.
   c) Go to step 8 only if you pressed **START**

Figure 6-54. Main Menu Calibration Screen after Sampling

8. Immediately analyze the sample offline for pH or DO.
9. Press **2** (Enter New Values) to display the following screen.

Figure 6-55. Probe Calibration Screen for Data Entry

9) Press ⬆ or ⬇ to move between the pH and DO fields. Press number keys to enter the offline pH or DO value in its corresponding field. Press **ENTER** to input the offline value.

10) After entering the value, press the following key depending on what you want to do next:
   - press **MONITOR** to leave the Calibrate mode
   - Press **ENTER** to perform another calibration

11) If you pressed **ENTER** in the previous step a screen similar to the following screen appears. It shows which probe was most recently calibrated (offline value was entered).
   - either `pH value written` (if pH was calibrated most recently)
   - or `DO value written` (if DO was calibrated most recently)
   - The elapsed time for `sample taken at` is when **START** was pressed for the last sampling prior to entering the offline value. This also is logged by the printer or ACULINK-DATA LOG, if in use.

   a) Press **1** to begin a new calibration of the pH or DO probe. Go to step 6.

   b) Press **2** to re-enter an offline value for a previous calibration. Only do this if an error occurred during the first entry of the offline value! In this case, the log will show Sample Taken once followed by two Calibration entries.

```
Calibrate:   1) Take Sample
             2) Enter New Values
pH value written    DO value written
sample taken at   0-00:03
```

Figure 6-56. Main Menu Calibration Screen after Data Entry

Notes:

1. **Blood gas analyzers are compatible with Luer-Lok syringes. If you have only a pH meter, you will need to modify the sampling method to accommodate the use of syringes during the offline measurement of the sample. Contact Account Services for details.**

2. **If a DO probe is in the cultureware but there is no offline equipment to measure DO, with the *PRE-BRX/DO* clamp open, calibrate DO to 140 mmHg weekly. After the calibration is complete, close the *PRE-BRX/DO* clamp and open the *POST-BRX/DO* clamp. It is more important to monitor post-BRX DO. When it drops below 100 mmHg, increase the speed of the CP by 100 ml/min, or more, unless it is at the maximum of 500 ml/min (single BRX) or 1000 ml/min (double BRX).**

Replacing Bottles

The attachment of medium, outflow, factor and harvest bottles is a necessary pre-inoculation and maintenance procedure. Use this procedure any time you have to connect bottles to the system.

Use sterile gloves and aseptic technique during bottle replacement. Work smoothly and quickly.

Procedure

1. Prepare the appropriate bottle (with **RESCU cap assembly**) to exchange with the bottle presently attached to the system.
2. Cover the connection between the media line and the cap assembly (or sterile protection) of the presently used bottle with sterile gauze saturated with disinfectant.
3. Sterilize the connection by soaking it for several minutes.
4. Meanwhile, cover the appropriate connecting port (see below) of the new bottle with sterile gauze saturated with disinfectant.

Outflow line	In port on bottle cap assembly
Harvest line	In port on bottle cap assembly
Media line	Out port on bottle cap assembly
Factor line	Out port on bottle cap assembly

5. Turn off the appropriate pump by setting the rate to zero using Override Controls.
6. On the appropriate line, close the clamp near the bottle or sterile protector.
7. After the connections are sterile, disconnect the tubing line from the cap assembly or protection. Keep the tubing from the flowpath covered with the sterile gauze at all times.
8. Remove the Luer plug from the new bottle and attach the line. Place the Luer plug in the old bottle's opening.

9. Open the clamp.
10. Clear the override (or return the pump rate to its previous override value).

Note: **Air may be removed from the Factor 3 tubing before it reaches the BRX and collects in the ECS. To do this, you will need to put a temporary clamp on the Factor 3 tubing downstream from the *FACTOR PRIME* port and attach a syringe to the *FACTOR PRIME* port. After the fresh Factor 3 bottle has been attached (steps 1–9 above), open the *FACTOR PRIME* clamp and set the Override Controls to run the Factor 3 pump at high speed until the air is in the syringe. Then, override the Factor 3 pump rate to zero, close the *FACTOR PRIME* clamp, remove the temporary clamp, and clear the override as described in step 10.**

11. Treat removed bottles appropriately. Outflow bottles should be decontaminated. All bottles should be washed as soon as possible.

Replacing LAVs and Septa

LAVs should be replaced after ~100 uses (or 96 hours of continuous use, i.e., when something has been connected to it long-term, which we do not recommend). Septa should be replaced after 7 – 10 samples.

1. Wear a mask, hair restraint, lintless lab coat, and sterile gloves. Aseptic technique is important in this procedure.
2. Check the sample line clamp to make sure it is closed.
3. Carefully wrap the old LAV or septum with gauze soaked in disinfectant. Wipe the entire area (tubing, LAV/septum and female Luer-Lok connector).
4. Holding the female Luer-Lok connector in one hand, carefully twist the old LAV/septum counterclockwise until it is loose. Do not remove at this time.
5. Open the sterile package of the new LAV or septum and hold the removable protector between your fingers.
6. Carefully remove and discard the old LAV or septum and replace it with the new item. Twist it clockwise until secure.
7. Sample site is once again ready for use.
8. Disinfect the surface of the new LAV and the inside of its cover. Install the cover on the LAV.
9. Suggest covering the septum with a disinfected cover, such as a piece of aluminum foil.

Removal of Cell Debris

Cells that slough off the BRX(s) are removed to some extent by the harvest pump, but most accumulate in the Expansion Chamber. Here, they can interfere with EC Cycling or disturb the environmental conditions within the ECS. For these reasons, the Cell Removal line is attached to the bottom of the Expansion Chamber. If followed regularly, the procedure described below will help you to minimize cellular buildup. An alternate method is provided after the standard method.

Note: **This procedure instructs you to remove the entire contents of the Expansion Chamber—cells plus ECS liquid—then return fresh serum-supplemented medium to the flowpath to avoid depleting the ECS of serum. Contact Account Services if you have any questions about modifying this protocol for serum-free cultures or for replenishing the ECS with conditioned medium.**

Standard Cell Removal Procedure

1. Needed supplies: at least two empty 60 ml syringes and possibly two 60 ml syringes filled with serum-supplemented medium and the usual supplies for aseptic sampling.

Note: **During this procedure you will be working with the incubator door open long enough to activate the temperature alarm. Also, your handling of the cultureware in step 7 may activate other alarms related to EC Cycling. In all of these cases, press *ALARM*, then *MONITOR* to silence the alarm until you have completed the entire procedure. Reset the alarms after this procedure is done.**

2. Press **E.C. CYCLE** then **2** to override EC Cycling to the Low Expansion Chamber volume.
3. Press **MONITOR** and wait until EC is approximately 108 ml.
4. Press **PUMPS** and override F3 and HP to 0 ml/hr.
5. Close the **EC RETURN** clamp.
6. Put a temporary clamp on the EC tubing that connects to the lower side port (in port) of the BRX(s), and close this clamp.
7. Disconnect the tubing line from the **EC** bulkhead port.
8. Aseptically attach an empty 60 ml syringe to the Cell Removal line.
9. Carefully shake the Expansion Chamber to loosen the cells and form a slurry.

10. Open the **CELL REMOVAL** clamp and remove as much of this suspension as possible.
11. Close the **CELL REMOVAL** clamp.
12. Aseptically replace the first syringe with an empty 60 ml syringe.
13. Repeat steps 10 and 11. Tilt the Expansion Chamber to the left to remove as much of the slurry as possible, if necessary.
14. Aseptically replace the second syringe with a 60 ml syringe filled with serum-supplemented medium.
15. Open the **CELL REMOVAL** clamp.
16. Inject the 60 ml of medium up the Cell Removal line and into the Expansion Chamber.
17. Close the **CELL REMOVAL** clamp.
18. Replace this syringe with the second syringe filled with serum-supplemented medium. Repeat steps 15 and 16.
19. Leave this syringe attached to the Cell Removal line.
20. Press **E.C. CYCLE** then **5** to clear the EC Cycle override.
21. Press **MONITOR**.
22. Remove the temporary clamp applied in step 6.
23. Reconnect the tubing line to the **EC** bulkhead port.
24. Open the **EC RETURN** clamp.
25. The cell removal procedure is finished.
26. Reset the alarms that may have been activated once they are between their respective Low and High Alarm Limits.
27. Optional: Process the collected cell slurry because it contains product from the ECS. Discard the cell pellet as a biohazard.

CAUTION: If the application has the potential to release a biohazardous agent, contact Biovest's Account Services department.

Alternate Cell Removal Procedure

1. Needed supplies: a sterile container (a bottle and cap assembly or a disposable bag) that holds ≥ 250 ml and can connect to the Cell Removal line's female Luer fitting.
2. Aseptically connect the sterile container from step 1 to the Cell Removal line.
3. Place the sterile container on the floor.
4. Close the **EC RETURN** clamp.
5. Carefully shake the Expansion Chamber to loosen the cells and form a slurry.
6. Open the **CELL REMOVAL** clamp.
7. Gravity alone may cause the liquid and cell slurry in the Expansion Chamber to flow into the sterile container. If not, press **E.C. CYCLE** then **2** to cause EC pressure to momentarily increase.
8. When the Expansion Chamber is reasonably empty, close the **CELL REMOVAL** clamp.
9. Press **5** to clear the EC Cycle override.
10. Press **MONITOR**.
10. Either leave the sterile container on the Cell Removal line, or replace it with a sterile male Luer plug or syringe.
11. Open the **EC RETURN** clamp.
12. Allow EC Cycling to resume. It will replenish the volume in the Expansion Chamber. If the MP rate is quite slow compared to the EC Cycling flow rate, using syringes to replenish the Expansion Chamber volume may provide better results.
13. The cell removal procedure is finished.
14. Reset the alarms that may have been activated once they are between their respective Low and High Alarm Limits.
15. Optional: Process the collected cell slurry because it contains product from the ECS. Discard the cell pellet as a biohazard.

PRE-INOCULATION PROCEDURES

After the flowpath has been flushed and filled with medium, it may be run without cells for a period of time before it is inoculated. This pre-inoculation phase of the culture is begun automatically after the Fill & Flush procedure, and it uses Process Control parameters for pump rates, cycling conditions, and pH control. You may use the default parameters for pH setpoint and pump rates, or you may change them to suit your needs. It is recommended that you change the default parameters for cycling (see below).

pH Calibration

During the pre-inoculation phase, the pH probe should be calibrated at least once to enable the system to establish the desired pH setpoint. If calibration is not performed, the software does not have an accurate basis for reading pH from the probe, and media components could be adversely affected by extreme pH created by an incorrectly monitored probe. Calibration at this time may also be helpful in detecting a probe that did not survive the autoclave conditions. A replacement can therefore be installed **before** the culture begins. Details on the calibration procedure are given in **Maintenance Procedures**.

DO Calibration

Although the system does not control dissolved oxygen levels in the media, calibrating the DO probe during the pre-inoculation phase permits the software to monitor DO readings accurately from the probe. This may prove helpful in detecting a probe that did not survive the autoclave conditions. A replacement can therefore be installed **before** the culture begins. Details on the calibration procedure are given in **Maintenance Procedures**.

EC Cycling

The conditions necessary to create EC cycling should be tested in the pre-inoculation phase. To do this, you will have to change the default delay time in Process Control to zero. To measure the actual Rise and Fall times, set the printer log time to a convenient interval (using SETUP, not Process Control).

Osmolarity

The effect of medium pump rate on osmolarity should be considered, especially during long pre-inoculation periods. Slow medium rates (<25ml/hr) have the potential of allowing the GEX to dehydrate the flowpath and consequently raise the osmolarity of the medium. This problem can be resolved by raising the medium pump rate temporarily. Contact Account Services if you have questions about this relationship.

Offgassing (Optional)

If the gas exiting the oxygenator has the potential of carrying biohazardous material from the cells or media, the off-gassing tubing should be installed. This is the black-collared Tygon tubing supplied with the instrument. It should be attached to the GEX OUT line and to the bulkhead fitting labeled "OUT". The barbed fitting on the rear of the instrument should be connected to slightly negative pressure and to a disposal system to ensure that gases are collected and properly treated.

Cytotoxicity Testing (Optional)

A few days before inoculation, samples of media should be removed from the ICS and ECS, then tested for cytotoxic residue. The cytotoxicity test is run to determine whether medium in the system has any toxic effect on cells.

All tests should be compared to medium which has previously been shown to be non-toxic. A certain percentage of cells will die even if the medium is not toxic. A known control will ensure that cell death is due to toxicity and not some other factor.

Flasks should be gassed aseptically with 5–10% CO_2 to prevent bicarbonate buffer in the medium from losing CO_2 until the flask is equilibrated with the incubator environment. While some cells survive this shock (loss of CO_2) which results in high pH, it usually induces a lag period of cell growth and some cells will die.

A high percentage of serum may bind toxins and mask a potentially bad lot of medium.

A suggested protocol follows. Other cytotoxicity tests may be used.

Cytotoxicity Testing Procedure

Run cytotoxicity assays of medium taken from the system against a control flask of the same type medium. All flasks should have the necessary nutrients at the same concentrations.

1. Label six T-25 flasks: two control flasks, two for ICS samples, and two for ECS samples.
2. Add 10 ml of control medium to each of the control flasks.
3. Remove two 20 ml samples, one from the IC circuit and one from the EC circuit, using the technique described in the sampling procedure.
4. Put half of the IC sample (10 ml) in one IC sample flask, and the other half in the second IC sample flask. Put half of the EC sample in one EC sample flask, and the other half in the second EC sample flask.
5. Add serum to all flasks so that the serum concentration is the same as that in static culture.
6. All of the flasks should have identical medium make-ups before proceeding to the next step.
7. Flush the flasks with 5 – 10% CO_2 in air.
8. Inoculate each flask with 5 x 10^5 cells with a viability of at least 85%.
9. Incubate in a 37°C, CO_2 incubator for two days.
10. Observe the flasks daily for cell number and viability. Include a count from the day that the flasks were seeded.
11. Test results may be determined in two ways:

First, a quantitative result may be obtained by counting cell number and cell viability in all of the flasks and recording the results. If there are no significant differences (based on customer pre-determined values), the system is ready to be inoculated.

Second, flasks may be observed carefully under a phase microscope and their appearance assessed in comparison to the controls. If the cell appearance in the sample flasks are as good as those of the controls, or better, the system passes the test and is ready for inoculation.

If the test results confirm cytotoxicity, **do not inoculate the flowpath module**.

- Continue to add medium to the system with EC Cycling in effect.
- Re-examine the flowpath for stagnant BRX or pinched lines.
- Contact Biovest International's Account Services. Be prepared with the lot number and manufacturing number of your cultureware, the conditions applied from Fill/Flush to the removal of cytotoxicity samples, and the specific cytotoxicity results.

Sterility Testing (Optional)

At the same time that you test for cytotoxicity, the system should be tested for sterility to be sure that you have not introduced bacteria or fungi into the system before inoculating it with your cell line. Draw samples from each of the ports used for cytotoxicity testing, and use the medium quality control procedures used previously (see **Medium Quality Control**).

Because sterility tests generally required several days to be completed, this test is used to double check the sterility of the system. If aseptic technique has been used throughout, the system should still be sterile. This test is simply a confirmation of that fact.

Serum Pre-treatment of the ECS

For cultures using serum-supplemented media, serum is added to the ECS at the end of the pre-inoculation phase to prepare the ECS with the same serum concentration as in static culture. This creates an environment similar to that in static culture, and minimizes the stress incurred from inoculating into a serum-free environment. Pre-treatment of the fibers can be done any time before inoculation, however, it is most effective if done between four hours and twenty-four hours before the inoculation

Because Biovest International's standard cultureware uses BRXs with a MWCO of ~60,000 daltons, media components with molecular weights lower than this may or may or may not freely exchange between the ECS and ICS.

An important consideration in the pre-treatment procedure is whether the components in the growth-supplemented EC media are smaller than ~60,000 daltons. If they are, it is possible they may pass to the IC. This dialysis can cause no or slow growth of the inoculum, in which case, the IC medium also can be supplemented.

The volume of serum required for pre-treatment depends on the concentration of serum used routinely in static culture, and it depends on the cultureware volume, which varies by bioreactor size and the number of bioreactor in the cultureware. Refer to Appendix A for system and ECS volumes in *MAXIMIZER* cultureware.

Notes:

1. **For other types of ECS pre-treatment, contact Account Services.**
2. **The serum pre-treatment protocol outlined here is optional and may be replaced with other pre-treatment procedures, depending on the type of medium and/or cells you are using. Consult Account Services if you have any questions.**

To perform the pre-treatment procedure, follow the steps outlined below. Refer to the procedures described in **Sampling**.

1. Aseptically fill a syringe with the desired volume of serum.
2. Aseptically fill a 3ml syringe with unsupplemented medium.
3. Disinfect the **FACTOR PRIME** port.
4. Attach the serum syringe to the **FACTOR PRIME** port.
5. When using the single BRX flowpath (600058-xxx):
   a) Open the **FACTOR PRIME** clamp
   b) Inject the serum
   c) Close the **FACTOR PRIME** clamp
6. When using the double BRX flowpath (600068-xxx):
   a) Put a temporary clamp on the tubing connected to the top EC port of one BRX.
   a) Open the **FACTOR PRIME** clamp
   b) Inject half of the serum
   c) Close the **FACTOR PRIME** clamp
   d) Move the temporary clamp to the tubing connected to the top EC port of the other BRX.
   e) Open the **FACTOR PRIME** clamp
   f) Inject the remaining serum
   g) Close the **FACTOR PRIME** clamp
   h) Remove the temporary clamp
7. Aseptically replace the empty serum syringe with the syringe containing unsupplemented medium.
8. Open the **FACTOR PRIME** clamp.
9. Inject the unsupplemented medium to flush residual serum in the tubing into the bioreactor(s).
10. Close the **FACTOR PRIME** clamp.

11. Leave the flush syringe on this port unless you are using sampling septa. This syringe can later be used to purge air out of the line when F3 bottles are replaced.
12. Be certain that the system is cycling properly. This will ensure that the serum is distributed throughout the BRX(s) and the Expansion Chamber. If you are using the double BRX flowpath, be sure that **both** BRXs are cycling properly but confirming there is no stationary air bubble in either of the two tubing lines that connect to the lower EC (side) ports.

Process Control Summary during Pre-Inoculation Phase

Single Bioreactor Flowpath: (600058-xxx, for either bioreactor size)

pH setpoint	7.2 (7.1 – 7.3 or the value determined from the cell line characterization)		
Temp setpoint	37.0 °C		
Alarms:			
pH	Low 7.10	High 7.50	
DO	Low *100	High 200	mmHg
Temp	Low 36.5	High 37.5	°C
Circulation pump	250–500		ml/min
Media pump	Basal	25 – 75	ml/hr
	Dilution	0	ml/hr
Base (F1)	Disabled		
EC Cycling	Delay	0-00:00	DDD-HH:MM
	Up	15	minutes
	Dn	15	minutes
	Transfer Vol	70	ml
Serum Control (F3)	Rate	0	ml/hr
	Delay	0-00:00	DDD-HH:MM
	ON	0-00:00	DDD-HH:MM
	Cycle	0-00:00	DDD-HH:MM
F2 Control (F2)	Rate	0	ml/hr
	Delay	0-00:00	DDD-HH:MM
	ON	0-00:00	DDD-HH:MM
	Cycle	0-00:00	DDD-HH:MM
Harvest	None (option 4)		

Double Bioreactor Flowpath: (600068-xxx, for either bioreactor size)

pH setpoint	7.2 (7.1–7.3 or the value determined from the cell line characterization)		
Temp setpoint	37.0 °C		
Alarms:			
pH	Low 7.10	High 7.50	
DO	Low *100	High 200	mmHg
Temp	Low 36.5	High 37.5	°C
Circulation pump	500–1000		ml/min
Media pump	Basal	25–75	ml/hr
	Dilution	0	ml/hr
Base (F1)	Disabled		
EC Cycling	Delay	0-00:00	DDD-HH:MM
	Up	15	minutes
	Dn	15	minutes
	Transfer Vol	70	ml
Serum Control (F3)	Rate	0	ml/hr
	Delay	0-00:00	DDD-HH:MM
	ON	0-00:00	DDD-HH:MM
	Cycle	0-00:00	DDD-HH:MM
F2 Control (F2)	Rate	0	ml/hr
	Delay	0-00:00	DDD-HH:MM
	ON	0-00:00	DDD-HH:MM
	Cycle	0-00:00	DDD-HH:MM
Harvest	None (option 4)		

INOCULATION PROCEDURE

The preceding part of this manual describes certain procedures that prepare and test the flowpath before cells are added to the BRX. Read them carefully, and be certain the necessary steps have been performed before continuing with this procedure. The last part in this section, **Optimization Procedures**, provides you with advice related to the inoculation of flowpath modules.

Note: **The following instructions are written for cells that have been grown in static culture using serum-supplemented media. Consult the Optimization Procedures part of this manual, or contact Account Services, if you have questions about using serum-free (or similar) media.**

Cells should be prepared so they will be in mid-log phase of growth on the day of flowpath inoculation. This can be determined by constructing a growth curve for suspension cells, or by selecting anchorage-dependent cells at the appropriate (typically 50 – 70%) confluency in static culture. To shorten the lag time of growth in the BRX, a minimum of 2×10^8 viable cells should be prepared for each bioreactor in the cultureware. The steps used to collect cells for inoculation should ensure the highest possible percent viability.

Resuspend the cells to 50 ml per BRX using a large bore (15 gauge) needle and the 60 ml syringes provided in the accessories package. Conditioned medium will probably provide the least stressful environment. Fill a small (10 ml) syringe with cell-free medium to flush the tubing after cells are injected into the BRX. Proceed immediately to the *MAXIMIZER*.

Note: **Even though the Outflow pump is running during the inoculation procedure, if you use inoculum volumes greater than 50 ml per BRX, you will run the risk of flooding the Integration Chamber. Watch this chamber's volume and do NOT allow it to fill faster than the Outflow Pump can remove volume!**

CAUTION: **If the application has the potential to release a biohazardous agent, contact Biovest's Account Services department.**

1. Press **START** then **INOCULATE**. All of the pumps and logging stop, and the software reminds you to make any necessary changes to Process Control. The most important change in Process Control is to set the EC Cycling Delay to a future time to ensure it does not activate immediately after completing the inoculation procedure, which could cause the inoculum to flow into the Expansion Chamber.

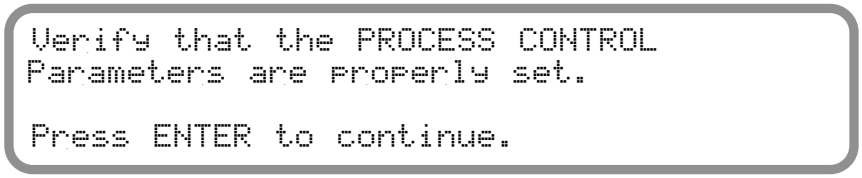


Figure 6-57: Instructions Screen 1 for Inoculation

CAUTION: **EC Cycling typically should not activate until approximately two to three days after inoculation for most applications. When using low- or protein-free medium in the ECS, longer delays may provide better results by giving the culture more time to become established before the start of EC Cycling.**

Note: **Perform the inoculation with minimum delays to prevent pH changing in the flowpath as a result of the stopped pumps.**

2. Press **PROCESS CONTROL**.
3. Press **ENTER** until `EC Cycling` appears.
4. Change `Delay` to `14-00:00`
5. Confirm the remaining Process Control settings are appropriate for a newly inoculated culture. Refer to the settings on page 6-80/81.
6. Press **START** then **INOCULATE** to return to the previous screen (Fig. 6-57).
7. Press **ENTER** to display the following screen.

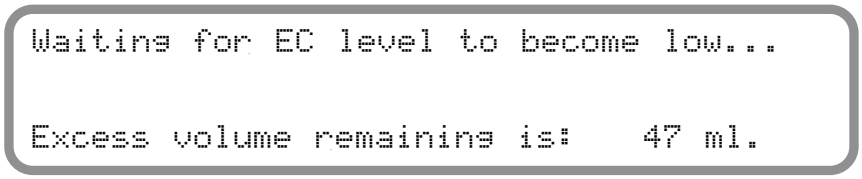


Figure 6-58: Instructions Screen 2 for Inoculation

8. EC pressure rises to lower Expansion Chamber volume to its low setting, 108 ml. The screen shows this excess volume being removed.

9. When the excess volume has been removed, the Outflow pump stops and the screen changes. Perform the onscreen instructions. For the instruction, `Remove IC Vent Gas Line`, disconnect the reusable Tygon tubing from the **IC** bulkhead port.

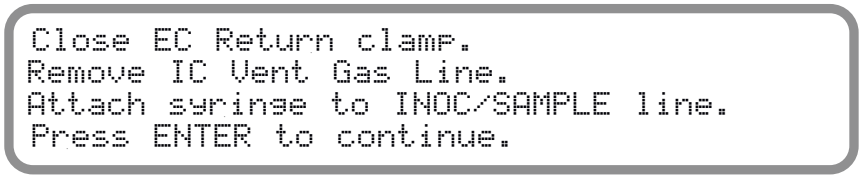


Figure 6-59: Instructions Screen 3 for Inoculation

10. The Outflow pump starts again, and the screen changes. For the instruction, `Open INOC/SAMPLE clamp`, open the clamp labeled **INOC/EC SAMPLE**.

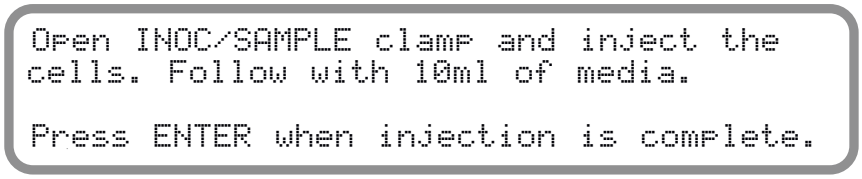


Figure 6-60: Instructions Screen 4 for Inoculation

11. If the cultureware has more than one BRX, apply a temporary clamp on the tubing that connects to the upper EC (side) port of the left-hand BRX to direct the inoculum into only the top of the right-hand BRX.

CAUTION: Do NOT clamp the top IC (end) port of the BRX!

12. Inject the cells. Excess fluid passes into the lumen and is removed at the Integration Chamber by the Outflow pump. As you inject cells, maintain constant pressure on the syringe, and rock the syringe gently to keep the cells suspended. Avoid adding any bubbles to the BRX. This step should require 1–2 minutes.

13. If the cultureware has two BRXs:
    a) Close the **INOC/EC SAMPLE** clamp
    b) Aseptically replace the empty syringe with one containing cells
    c) Move the temporary clamp to the tubing that connects to the upper EC (**side**) port of the right-hand BRX to direct the inoculum into only the top of the left-hand BRX. See *CAUTION* note above!
    d) Open the **INOC/EC SAMPLE** clamp and repeat step 13
    e) Close the **INOC/EC SAMPLE** clamp
    f) Remove the temporary clamp

14. When the BRX(s) have been inoculated, replace the last empty syringe that had cells with the 10 ml syringe containing cell-free medium.
15. Open the **INOC/EC SAMPLE** clamp.
16. Inject the medium to flush any residual cells in the tubing up and into the BRX(s).
17. Close the **INOC/EC SAMPLE** clamp.
18. Press **ENTER** to advance to the following screen.

Figure 6-61: Instruction Screen 5 for Inoculation

19. For the instruction, `Close the INOC/SAMPLE clamp`, close the clamp labeled **INOC/EC SAMPLE**.
20. Replace the flush syringe with a new, sterile syringe, not the green plug, which is no longer provided as an accessory with the cultureware.
21. Press **ENTER** to display the following screen. This also causes the Outflow pump to stop.

    Note: Do not open the **EC RETURN** clamp at this time. EC Cycling is inactive at this time, so this clamp does not need to be open, and it is safe to leave this clamp closed. The **EC RETURN** clamp will be opened when EC Cycling is activated, see EC Cycling information on page 6-95.

    `Attach IC Vent Gas Line` by connecting the reusable Tygon tubing to the **IC** bulkhead port.

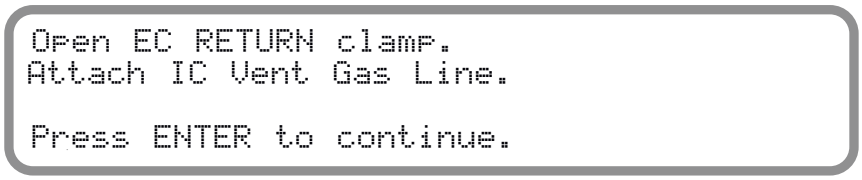


Figure 6-62: Instructions Screen 6 for Inoculation

22. Pressing **ENTER** displays the following screen, which briefly appears to indicate the inoculation procedure is finished.

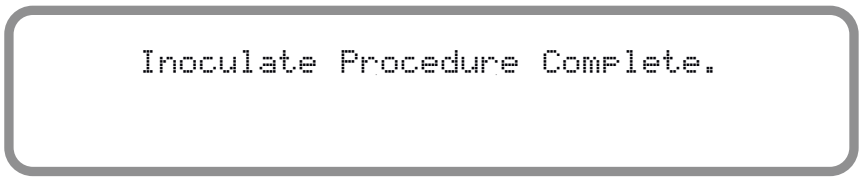


Figure 6-63: Completion Screen for Inoculation

23. The following screen appears and Process Control automatically activates before **ENTER** is pressed.

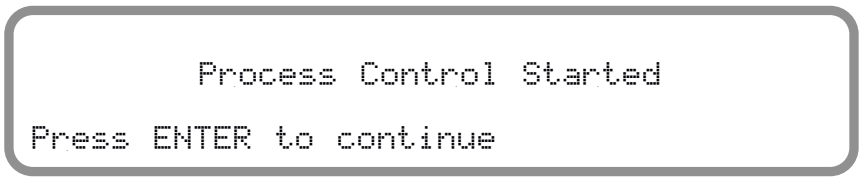


Figure 6-64: Final Screen for Inoculation

24. Press **ENTER** to leave this last Inoculation screen.
25. The Monitor screen appears. The following actions have automatically occurred:
    - Elapsed time has reset to `0-00:00`
    - `INOC` has ended and `RUNNING` is the active mode of operation
    - Active Process Control is active (Active Process Control LED is on), so logging, alarms, setpoint control and other activities are now active
26. Press **ALARM** to silence the audible alarm and deactivate the external alarm relay. Alarms normally occur (such as temperature, pH and DO) immediately when mode `RUNNING` mode begins. Review the alarms stored in the queue to ensure unusual alarms are not present.
27. Press **MONITOR** and watch temperature, pH and DO readings. After approximately 20 minutes they will be equilibrated and likely within the user-defined `LOW` and `HIGH` alarm limits as defined in Process Control.
28. Press **ALARM**. Individually restore each stored alarm by using arrow keys to locate each parameter that is in its alarm range and pressing **ENTER** to restore its alarm. Press **MONITOR**.

29. You may wish to:
    - record the time of day that corresponds to the new elapsed time of 0-00:00 to know when the inoculation was completed.
    - take time-zero IC sample of medium to establish baseline levels of metabolites. Wait at least about 30 minutes after inoculation before removing this sample to allow their concentrations to stabilize.

Note: **Process Control sets the Gas # at 7 after Inoculation. Gas # gradually increases during the first 15–20 minutes to establish pH control. pH may go out of range while pH control is determing the correct Gas # to use, causing a pH out of range alarm. Follow steps 25–27 to respond to the alarm.**

POST-INOCULATION PROCEDURES

After inoculation, the culture will require periodic changes to the feed rate, factor rate, harvest rate, cycling conditions, and circulation rate. Although some of these parameters are automatically set into Process Control, *you* must perform manual changes to others. Information useful to these procedures was provided earlier in the descriptions on **Oxygen Delivery, Feed Strategy,** and **Harvesting Strategy**. In addition, the last part of this section, **Optimization Procedures**, contains more ideas on "customizing" various parameters to your cultures.

After a culture has been inoculated, various system functions need to be examined to ensure that a healthy culture is maintained. The following schedule is suggested for daily and periodic checkouts of the culture.

Note: **You may wish to prepare a printed form to remind you of these items, as well as to maintain a record of activities during each run. Contact Account Services for ideas and examples of this type of documentation.**

Daily

- EC Cycling is inactive after inoculation. Remember to activate it when necessary, see page 6-95
- Sample for metabolite and product assays and determine which pump speeds to change based on that information
- Check the circulation pump to ensure that it is operating smoothly and does not have leaks
- Check the fluid in the Integration and Expansion Chambers to verify that the level is satisfactory and for signs of contamination
- Check the IC and EC chamber vent filters to ensure proper gas line connections (especially after inoculation and removal of cell debris)
- Inspect the outflow and feed bottles for proper fluid levels
- Check CO_2 tank and optional backup air supply
- Check ALARM screen and correct problems as needed

Periodically

- Calibrate pH and DO probes
- Replace the feed, factor(s), outflow, and harvest bottles
- Replace sampling port septa
- Remove unwanted cell debris from Expansion Chamber

End of Culture Procedures

The following is a description of the procedures that are to be performed after a culture run is finished. Instructions will be given for removing the cultureware, discarding the disposable components, and saving the reusable components. A separate part of Section 6, **Contamination**, will discuss the details of handling cultureware affected by contamination.

Shutdown Procedures

Perrform the following steps to terminate the active culture run.

1. If ACULINK-DATA LOG, is in use, export the log and save it. Shut down the computer and turn off its power.
2. Press **HOLD** then **2**.

Figure 6-65: Selection Screen for HOLD key

3. The instrument sends *Process Aborted* to the printer, if Logging is enabled. (ACULINK-DATA LOG, does not receive the *Process Aborted* log entry.)
4. If a printer is in use, retain the printout. Turn off power to the printer.
5. Close the CO_2 supply to the instrument.
6. Backup Air is optional. If it is in use, close this supply to the instrument.
7. Turn off power to the instrument to minimize wear and tear to its components.

Recovery of Harvest

After the run is terminated and the Circulation Pump speed is 0 ml/min, you may, if desired, manually empty the Expansion Chamber and BRX(s) to collect their remaining supernatant. These are just two methods to do this. Other methods also are possible, such as using multiple syringes connected to various ports. The effectiveness of any method depends on the accumulated cell mass. If product titer was low when the run was terminated, this harvest recovery process is not likely worthwhile.

Use the EC Cycling Override:

1. Put clamps on the IC inlet and outlet tubing of the BRX(s) (to prevent unwanted IC medium from also being collected).
2. Aseptically connect the F3 tubing to a Harvest collection container
3. Remove the F3 tubing from the pump head
4. Close these clamps:
   a) **INOCULATE/EC SAMPLE**
   b) **HARVEST**
   c) **FACTOR PRIME**
   d) **CELL REMOVAL**
   e) Lower **CHECK VALVE BYPASS**
5. Open these clamps:
   a) **FACTOR 3**
   b) **EC RETURN**
   c) Upper **CHECK VALVE BYPASS**
6. Override EC Cycling to Drain
7. Collect supernatant from BRX(s) and proceed when the ECS is empty
8. Close the upper **CHECK VALVE BYPASS** clamp
9. Open the lower **CHECK VALVE BYPASS** clamp
10. Collect supernatant from Expansion Chamber. Proceed when it's empty
11. Press **HOLD** then **2**
12. Close all clamps and process the supernatant

Use the Factor 3 Pump:

1. Put clamps on the IC inlet and outlet tubing of the BRX(s) (to prevent unwanted IC medium from also being collected).
2. Remove the F3 pump segment from the pump head
3. Reverse-load the F3 pump segment in the pump head (in the orientation the Harvest pump segment is loaded).
4. Aseptically connect the F3 tubing to a Harvest collection container
5. Disconnect the reusable Tygon gassing line from the vent filter on the Expansion Chamber.
6. Close these clamps:
   a) **INOCULATE/EC SAMPLE**
   b) **HARVEST**
   c) **FACTOR PRIME**
   d) **CELL REMOVAL**
   e) Lower **CHECK VALVE BYPASS**
7. Open these clamps:
   a) **FACTOR 3**
   b) **EC RETURN**
   c) Upper **CHECK VALVE BYPASS**
8. Override F3 Pump to 400 ml/hr
9. Collect supernatant from BRX(s) and proceed when the ECS is empty
10. Close the upper **CHECK VALVE BYPASS** clamp
11. Open the lower **CHECK VALVE BYPASS** clamp
12. Collect supernatant from Expansion Chamber. Proceed when it's empty
13. Press **HOLD** then **2**
14. Close all clamps and process the supernatant

Removal of Cultureware Components

The cultureware has disposable and reusable components. Read the following procedures, and be certain that you save the reusable components for use in future cultures.

CAUTION: If the application has the potential to release a biohazardous agent, contact Biovest's Account Services department.

1. Close all clamps, particularly Outflow, Media, F1, F2, F3 and Harvest.
2. Disconnect the In, Out (if used), IC, and EC gassing lines from their bulkhead and cultureware connections. **Save them for the next run!**
3. Disconnect all electronic cables from their bulkhead jacks.
4. Remove the temperature cable and DO probe cable (if it's in use). **Save them for the next run!**
5. For the reusable pH probe: Remove the cable. **Save it for the next run!**
6. For the disposable pH probe:
   a) the cable, case and the probe are discarded, so leave them in the cultureware as they are.
   b) remove the reusable plastic pH holder. **Save it for the next run!**
7. Remove the two metal screws and plastic knob from the circulation pump bracket and crank pin. **Save them for the next run!**
8. Slide the cultureware's circulation pump out of the metal bracket and off of the crank pin. The cultureware's circulation pump has a cylindrical bearing that should slide off of the crank pin. If it stays on the crank pin, remove it too because it is discarded with the cultureware.
9. Remove the six tubing lines from the pump heads.
10. Set the six pump heads and their screws aside.
11. Lift the cultureware up and over the metal circulation pump bracket and pull it out of the incubator.
12. Open a biohazard bag and place it on a benchtop.
13. Place the cultureware on the opened biohazard bag. **Do not close it yet!**

14. Remove the reusable pH probe case assembly and the DO probe case assembly (if in use) from the cultureware:
    a) put temporary clamps on the two tubing lines that connect to the pH probe case
    b) put temporary clamps on the two tubing lines that connect to the DO probe case
    c) disconnect the tubing from the probe cases and
    d) reconnect the cultureware's open pH and DO tubing lines by installing the fittings saved from Cultureware Sterile Assembly (see on page 6-37).
    e) remove the temporary clamps
    f) drain the probe case assemblies
    g) remove both probe case assemblies and set them aside
    h) coil the long tubing lines and place them on top of the culutreware
    i) seal the biohazard bag and dispose of it as a biohazard
15. **Clean the pump heads!** They likely will have small pieces of pump tubing in their grooves and other places. They also may have media spills in them.
16. Each pump head requires two white, teflon washers. Replacements must be installed before the next run. See Appendix B, **Ordering Information**.
17. Once the pump heads are clean and reassembled, store them or reattach them with the mounting screws.
18. Reconnect to the bulkhead jacks: the reusable pH cable, the DO cable (if in use), the temperature probe, and the four gassing lines.
19. Clean the reusable pH probe and the DO probe case assemblies:
    a) remove the probes from their cases
    b) do not touch the tip of the DO probe
    c) briefly rinse the probes with water
    d) put the pH probe in its storage bottle with fresh storage solution (see Appendix B)
    e) screw the electrical cap on the pH probe
    f) put the protective cap on the tip of the DO probe and screw on its electrical cap
    g) disassemble the probe cases
    h) discard the o-rings: 3 for pH, 1 for DO (cultureware includes new ones)

i) rinse the probe case pieces with water and clean with tissue culture-grade detergent and rinse with deionized water

j) save both probe cases for the next run

k) store the pH probe so its tip remains wetted in storage solution

l) store the DO probe (preferably vertically, with the tip at the bottom)

Notes:

1. **After a culture has been terminated, do not autoclave-sterilize the pH and oxygen electrodes until they are prepared immediately before the next run. Excessive autoclave cycles will shorten their lifespans and/or reduce their effectiveness. If the flowpath is contaminated or a biohazardous agent may be present, contact Biovest's Account Services department.**

2. **Do not forget to remove the temperature probe from the cultureware. Autoclaving it will damage it!**

- Properly dispose of the contents of all bottles that were attached to the flowpath. Remove the cap assemblies (and any labels) from the bottles, and clean as described in **Sterilizing Bottles and Cap Assemblies**.
- Clean the incubator and cabinet of the *MAXIMIZER* with a disinfectant or mild soap solution. Be careful to not allow disinfectant liquids to wick behind the keypad or enter the instrument in other locations! Avoid spilling any solutions on the circulation pump rotor, in the bulkhead connections and in the incubator circulation vent.

OPTIMIZATION PROCEDURES

The following suggestions are offered to users to improve the quality of cultures grown in the *MAXIMIZER*. Because each cell line responds individually to culture conditions, it is not possible to specify protocols that will work in all situations. However, this information will give you some ideas to consider. Experience with using these suggestions is usually the best method of instruction.

Biovest International's Account Services personnel are always available to assist you in optimization and will be happy to answer your questions as they arise.

Inoculation

Several factors related to the inoculum influence the growth of the culture in the flowpath. These include the number of cells per BRX, their viability, and the stage of growth when the inoculum is prepared.

- A higher inoculum population will shorten the period between inoculation and indication of significant metabolism. Although this may seem to be an advantage, the user must be aware of the practical concerns in preparing a higher density inoculum:
  - How many static culture vessels are needed?
  - What volume of cell suspension can be processed safely?
  - How much time is required?
- Anchorage dependent cells may require a higher inoculum density than suspension cells. Re-suspending them in conditioned medium rather than fresh medium may improve the initial growth period by providing the cell-specific growth factors secreted by the cells.
- Best results are often obtained by preparing the inoculum from cells that are in mid- to late-log phase of growth. A standard growth curve is often useful in scheduling the progress of cells in static culture to coincide with the day of inoculating the flowpath.

- An option when inoculating is to split the inoculum and inject half of the cells into the upper EC port, which is the normal port, and the other half of the cells into the lower EC port (via the Factor Prime port). This may lead to better distribution when the culture's expansion begins. This effectiveness depends on several factors, including how sticky the cells are and how much protein is in the EC medium, among others.
- Although the cultureware permits several options for adding cells, this manual has described the simplest method. Using a pressurized bottle to deliver cells to the BRXs may be dangerous if the bottle is weak or damaged. It is possible to distribute cells along the entire length of the BRX by circulating a cell suspension with a vented bottle and peristaltic pump, but this process requires more time than the standard technique, caution must be observed when using the override functions to control pump rates, and the longer inoculation time and pump action may cause cell damage.
- Forcing excess media from the ECS to the IC is sometimes called a transmembrane method of inoculation. Depending on the pore size and total surface area of the BRX(s), this method may cause a temporary pressure buildup (back-pressure) within the ECS. If the EC CHAMBER RETURN clamp is opened immediately after this type of inoculation, the back-pressure will cause a short burst of media to flow from the ECS of the BRX(s) to the Expansion Chamber. To avoid displacing cells in this manner, keep the Expansion Chamber clamps shut until process control has begun, manually vent the Integration Chamber to zero (room) pressure, and then open the Expansion Chamber clamps. Replace the vent line to the Integration Chamber after a few seconds.

- Seeding a BRX more than once may be considered an option in the following situations: low inoculum density and/or viability, poor initial growth, re-use of the flowpath. Ideally the first situation should be avoided, but time constraints or other factors may take precedence over preparing a suitable inoculum. In the other two situations, it may be best to flush the cells out of the BRXs before re-use, although cleaning a BRX that was densely populated is usually difficult and may not be recommended because of manufacturing regulations involving the desired product. Poor initial growth in the *MAXIMIZER* culture may be attributed to many conditions that can be avoided before inoculation.
    - Cytotoxicity should be assessed before cells are added to the BRXs. The procedure for this type of evaluation is described in **Cytotoxicity Testing**.
    - Characteristics of cells in static culture will give some indication of how fast they will appear to grow in *MAXIMIZER* cultures. Cells that exhibit relatively slow growth in static culture will respond similarly in perfusion systems.
    - Problems associated with microbial contamination may be avoided by using antibiotic-free media in static culture and by routinely testing stock cultures for mycoplasma and unwanted viruses.

Circulation Rate

As a culture grows, cells consume more of the oxygen dissolved in the surrounding medium and therefore need a greater delivery to maintain the same level of available oxygen. By increasing the circulation pump rates, the flowpath is capable of delivering more dissolved oxygen from the IC (saturated with oxygen by the gas exchanger) to the cell mass. If cultures require dissolved oxygen (for growth or production), it is best to increase the circulation pump rate gradually and allow 1–2 hours before measuring any changes in dissolved oxygen.

EC Cycling

EC Cycling should be activated when the cell culture shows signs of steady metabolic growth — a period of two to three days of glucose concentration decreases or glucose uptake rate increases, lactate concentration or lactate production rate increases, etc.

Typically, EC Cycling will begin some time during the first week after inoculation of the bioreactor(s).

To activate EC Cycling:

1. Open **EC RETURN** clamp.
2. Press **PROCESS CONTROL**.
3. Press **ENTER** until the following screen appears.

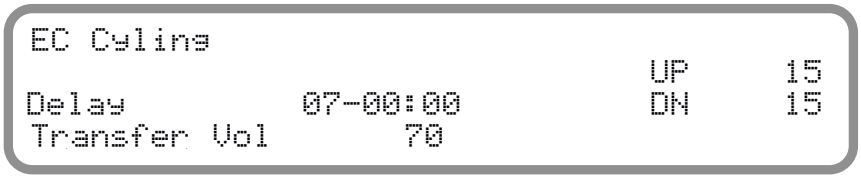


Figure 6-66. EC Cycling Parameters Screen

4. Use ⬅⬆➡⬇ to move the cursor between the four programming options.
5. Change `Delay` to `0-00:00` (by pressing **0** once and pressing **ENTER**).
6. Press **MONITOR** to see `Cycle` state change from `RISE` to `FALL` according to the `UP` (EC Chamber volume increases duration) and `DN` (EC Chamber volume decreases duration) times in minutes.

Routine EC media have enough protein supplementation that the default `UP` and `DN` times of `15` minutes work well for the entire run. EC media with low or no

protein supplementation may need to use 60 – 90 minute UP and DN times for the first few days when EC Cycling is active. When this strategy is followed, decrease these times by 15 – 30 minutes every few days. Continue this step-wise reduction until the default UP and DN times of 15 minutes are in use unless metabolic activity worsens. In such cases, decrease these times more slowly. Once the bioreactor contains areas of high cell density, UP and DN times of 15 minutes are important to maintain maximum culture viability and productivity.

The default Transfer Vol (transfer volume) is 70 ml, which normally works well when using various EC media, for the entire run, and when using cultureware with one bioreactor.

The default Transfer Vol of 70 ml is sufficient for the first approximately two weeks of active EC Cycling time when using cultureware with two bioreactors. After this time it may be beneficial to increase the transfer volume in steps of 15 – 35 ml every 2 – 3 days until the maximum transfer volume of 140 ml is in use, unless metabolic activity worsens. In such cases, increase the transfer volume more slowly.

Reducing the initial transfer volume may be necessary especially if the EC medium is protein-free or low in protein because the cell culture may require a longer period of time to become established among the bundle of hollow fibers. When this strategy is followed, increase the transfer volume in step-wise amounts of 15 – 35 ml every 2 – 3 days until the maximum suggested transfer volume of 70 ml is in use unless metabolic activity worsens. In such cases, increase the transfer volume more slowly.

Harvesting

- For secreted products that show feedback inhibition, the user should determine the threshold level to monitor before starting the harvest pump at a time different from the default value. Samples analyzed from the flowpath will provide the necessary information in this case.
- After the harvest pump has been started, product will be collected and measured for content. Depending on the type of product secreted by the cells, this measurement may be in units of concentration or activity. The harvest rate may be gradually increased until the product levels reach a satisfactory concentration/activity, or until the daily yield reaches a maximum amount, or until the harvest volume becomes difficult to process. The previous discussion on feedback inhibition should also be considered when pump rates are changed.
- A comparison of secreted product from the harvest bottle and the sample port will undoubtedly show different levels (concentration or activity) of harvested material. This happens because the harvest bottle contains an "average" of product material from the ECS throughout a specified period, while the sample originates from a single point in time. In addition, product levels in the sample may vary with the phase of cycling. Therefore, to measure accurately from the flowpath, samples should always be removed at the same time during the cycling process.
- Cooling the harvest bottle is a good way to preserve product integrity by preventing thermal degradation. It also has the advantage of stabilizing the changes in pH due to harvested cells, as well as reducing the activity of proteases released by cell lysis. If cell buildup in the harvest becomes a concern, the bottles should be replaced as often as possible, and the contents separated by centrifugation.

Serum Requirements

The pre-treatment of BRX ECS with serum is an important step in conditioning the flowpath before inoculation. Although the inoculum should contain the same amount of serum as in static culture, lack of serum in the EC would dilute the inoculum to an undesirable level. Therefore, serum should be added to the EC prior to inoculation. This procedure is described in **Serum Pre-treatment of the ECS**.

- Some components of serum are consumed while others (notably enzymes) are used as tools for biochemical reactions. The serum pump should be started when either of these functions/components is exhausted. Monitoring growth and productivity parameters will give an indication of the need to add serum.
- Cultures that show a drop in metabolic activity may require additional serum to recover. A syringe can be used to inject a quantity of pure serum into the EC.
- However, as a culture matures, it becomes less dependent on the serum. To reduce the serum content of the ECS, two procedures may be used. The factor bottle may be replaced with one that has less serum, or the factor pump rate may be lowered. These changes should be performed gradually to avoid sudden changes in environmental conditions. Metabolism and productivity should be monitored to determine the effect of lowering the serum content.
- If a culture does not begin expanding and consuming nutrients for many days after inoculation, an option to encourage their growth is to add growth supplement to the IC medium at the same concentration as what is in the EC medium (or switch to serum-free media in the IC if it is the EC medium and FBS is not in use). Continue this until the culture has expanded for one to two weeks. Change directly to unsupplemented IC medium at that time, or if necessary, periodically reduce the concentration (in steps) of growth supplements in the IC medium. This scenario can eliminate a dialysis (loss) of low molecular weight components from the ECS that was causing the culture to remain in lag phase. Once the culture has been growing for many days, it may continue to expand when IC medium is unsupplemented.

Serum-free Media

Experience with serum-free media varies widely. A culture can be started with serum-supplemented medium, then weaned onto serum-free medium, or it can be started with serum-free medium. Another technique is to add serum-free medium only to the EC. Regardless of the technique, the important point to consider is that the large molecular weight components of serum-free medium must be added directly to the EC.

Hybridomas grown in serum-free medium may not remain in the BRX if the protein content of the serum-free medium is too low. In this case, cycling and harvesting conditions should be modified to avoid excessive loss of cells.

SOFTWARE

If you have a problem that appears to be related to the software, verify the following items before notifying Account Services:

1. Are Override Controls in use, and are they performing as expected to supersede Process Control? Have you properly removed an override, or is it still active?
2. Determine which revision level of firmware your instrument is currently using. This may be done by checking the PROMs list; to do this, press SETUP, then choose menu item 6.

Alarms

The *MAXIMIZER* is capable of generating 33 alarms. At the time an alarm condition occurs:

- Audible alarm makes a 1-second tone every 4 seconds
- External alarm relay closes
- Alarm message and details (see next page) are recorded by ACULINK-DATA LOG or the printer, if either is in use.

Therefore, when an alarm occurs, common practice should be to:

1. Press **ALARM** to see what happened
2. Press **MONITOR** to silence the alarm and deactivate the external alarm relay
3. Resolve the alarm condition
4. Press **ALARM** and use the arrow keys to display the desired stored alarm
5. Press **ENTER** to restore the alarm so it can be activated in the future

See chapter 5, section ALARM Key for further information about the information in alarm screens and responding to them.

Each of the 33 alarm messages has the same format. Each alarm has a:

- Alarm name
- Alarm#: a unique number for each alarm name
- data1: variable number for each alarm message and alarm condition *
- data2: variable number for each alarm message and alarm condition *
- elapsed time: when alarm condition occurred (Days-Hours:Minutes format)

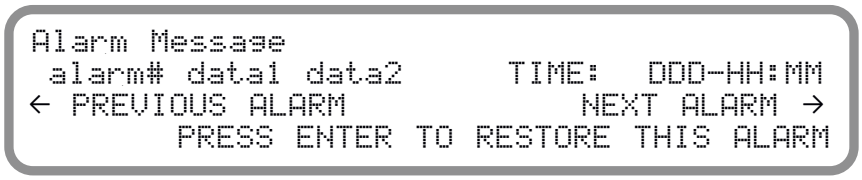


Figure 7-1: General Format for Alarm Screen

* data1 and data2: the meaning of these values is not detailed in this manual. They are used by Biovest personnel to provide technical assistance or during field service. If you report an alarm condition to Account Services, please include all of the displayed information.

Alarms 34, 35, and 36 monitor internal instrument functions. Even when they are stored in the alarm queue, each new occurrence of these alarms causes another activation of the audible alarm and external alarm relay, and it is recorded recorded by AcuLink-Data Log or the printer. If these alarms occur more than once in a short period of time, contact Biovest's Account Services.

The remaining alarms do not re-activate the alarm responses, if they are stored in the alarm queue.

Some information about each of the 33 alarms is presented below. Some of alarm descriptions will be useful to you, but they are intended for qualified service personnel.

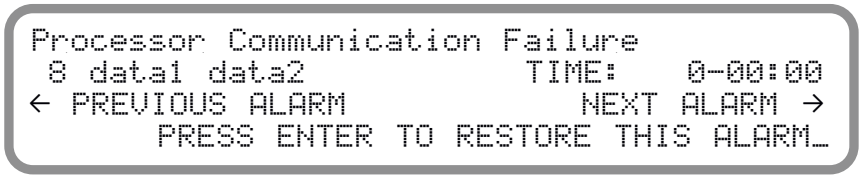


Figure 7-2: Format for Alarms 1 Through 8

Alarms numbered 1 – 8 indicate eight types of failures of the main control program to communicate with secondary programs. If this alarm occurs once, it may be of little concern, because it could be caused by electrical noise. However, repeated occurrences need **immediate** attention. The primary causes are improper DIP switch settings on the pump or monitor boards, loose or faulty wire assemblies, or a malfunction in various circuit boards. This problem likely requires repair service.

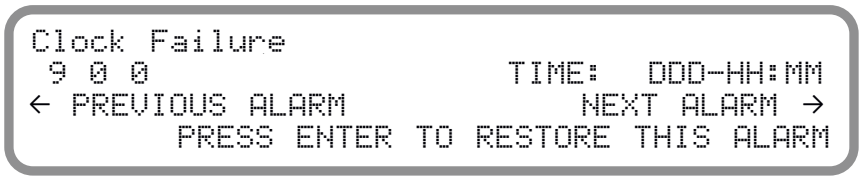


Figure 7-3: Alarm Screen 9

This alarm means a failure of the chip on the control board, and it must be replaced by service personnel. When the clock fails, all operations related to elapsed time will not function (e.g., process control, circulation).

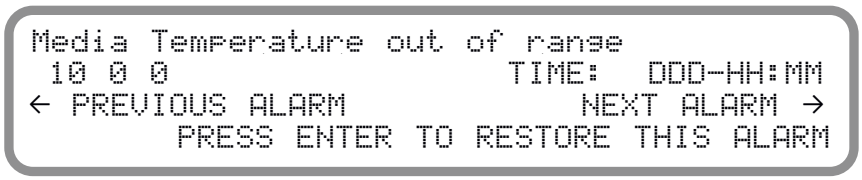


Figure 7-4: Alarm Screen 10

This alarm means the media temperature is outside of the limits set in Process Control. This may be due to recent activity inside the incubator, a fault in the heating system, neither of which is user-serviceable.

Users can check the following items to try to correct this problem:

- Incubator door fully latched on its pin? Is the gasket around the door completely sealing the incubator against air leaks?
- Sufficient clearance at the rear of the instrument for air to circulate through the inlet and outlet fans?
- Temperature cable fully connected in the cultureware and is the insulation covering the sensor tip?
- Temperature probe fully connected in its bulkhead jack?
- Room temperature within the operating specifications (18 – 26°C)?
- Does another temperature probe fix the problem?

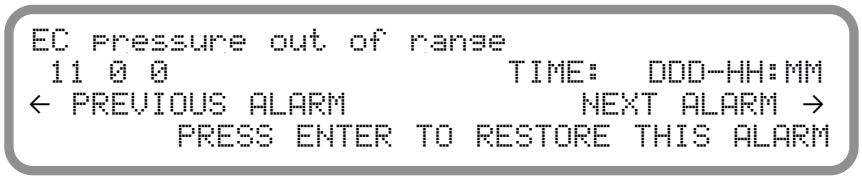


Figure 7-5: Alarm Screen 11

This alarm means the pressure in the Expansion Chamber is >280 mmHg. Verify that the chamber is not flooded and its gassing line is attached properly. This problem likely requires repair service.

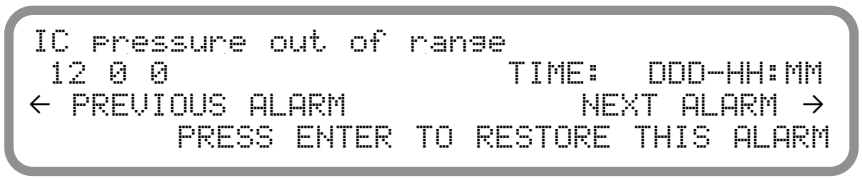


Figure 7-6: Alarm Screen 12

This alarm means the pressure in the Integration Chamber is >280 mmHg. Verify that the chamber is not flooded and its gassing line is attached properly. This problem likely requires repair service.

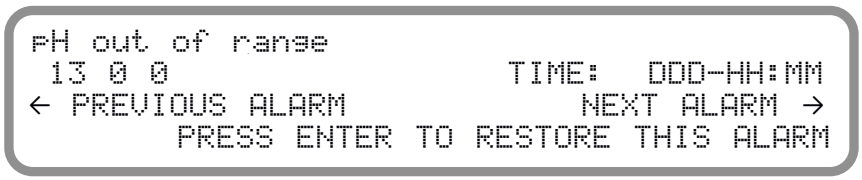


Figure 7-7: Alarm Screen 13

This alarm means the current pH value is outside of the alarm limits set in Process Control. Verify the following items to try to correct this problem:

- Bulkhead IN port connected to GEX IN vent filter port?
- Wet GEX Vent filter?
- GEX Out clamp open? Gas lines to and from GEX pinched or plugged?
- Off-gas port at the instrument's back panel not obstructed or plugged?
- CO_2 gas line to the instrument and its gas supply pinched, disconnected, closed?
- CO_2 gas supply properly set to deliver 15 ± 1 psi?
- pH cable properly connected to the probe and bulkhead jack?
- pH probe A/D reading within acceptable range? (See Section 6, **pH Probe Evaluation**.)
- Calibration of the pH probe fixes problem?
- Calibration procedure being properly performed?
- Circulation pump turning smoothly?
- Bubbles in pH probe case?
- Bubbles in the bioreactor(s) or in the IC?
- Medium Dilution set high enough to sufficiently dilute lactate?
- Base Addition attempted?

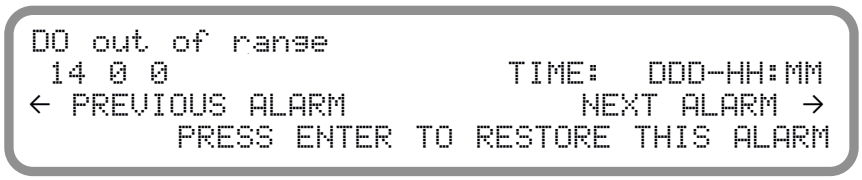


Figure 7-8: Alarm Screen 14

This alarm means the current DO value is outside of the alarm limits set in Process Control. Verify the following items to try to correct the problem:

- Bulkhead IN port connected to GEX IN vent filter port?
- Wet GEX Vent filter?
- GEX Out clamp open?
- Gas lines to and from the GEX pinched or plugged?
- Off-gas port at the instrument's back panel not obstructed or plugged?
- DO cable properly connected to the probe and bulkhead jack?
- DO probe A/D reading within acceptable range? (See Section 6, **DO Probe Evaluation**.)
- Calibration of the DO probe fixes problem?
- Calibration procedure being properly performed?
- Circulation pump turning smoothly?
- Bubbles in pH probe case?
- Integration Chamber vent filter wet and thereby creating an over-pressurized system?

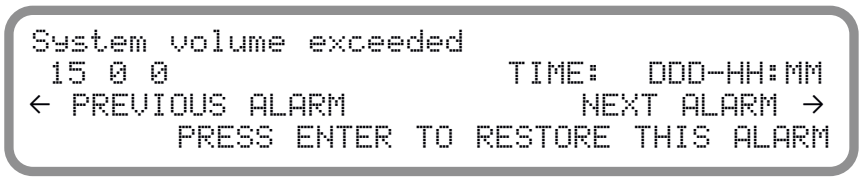


Figure 7-9: Alarm Screen 15

This alarm means the Expansion Chamber is approaching its maximum volume.

Take immediate action!

1. Inspect the IC and EC chambers.
2. Press **HOLD** if either chamber is about to, or has, flooded.
3. If either chamber's filter is about to become wet:
   a) Close **IC** (Vent) clamp if the IC vent filter is in jeopardy.
   b) Put a temporary clamp on the tubing between the Expansion Chamber and its vent filter if the EC vent filter is in jeopardy.
   c) Close **EC RETURN** clamp if the EC vent filter is in jeopardy.
   c) **Prevent these filters from getting wet!** If this happens, the filter likely must be replaced with a new, sterile filter. Do this aseptically!
4. If the filter is wet, disconnect its corresponding gassing line from the bulkhead port to prevent (additional) medium from flowing into the instrument! If this happens, the instrument may require service. Inspect the IC or EC port for the presence of cell culture medium.
5. Drain IC chamber:
   a) If **HOLD** was pressed, press **1** first
   b) Override Outflow pump to maximum, 4230 ml/hr
   c) Override Media pump to 0 ml/hr
   d) Clear overrides when Outflow pump pumps air from IC chamber.
6. Drain EC chamber:
   a) Remove medium via the Cell Removal line using syringes — or—
   b) If IC chamber volume is OK:
      - override Outflow pump to 4230 ml/hr
      - connect EC gassing line to EC chamber and instrument
      - override EC Cycling to Low
      - clear overrides when EC volume is ~108 ml

Contact Biovest's Account Services for technical assistance

Verify the following items to try to correct the cause of the System Volume Exceeded problem:

- Any clamps incorrectly open or closed?
- Any tubing pinched or plugged?
- Vent lines fully connected to the IC and EC chambers and bulkhead ports?
- Vent lines attached to the correct bulkheads, i.e., IC to IC and EC to EC?
- Any unintended pump overrides still active, particularly Outflow Pump?
- EC Chamber ultrasonics cable fully connected in its bulkhead jack?
- Outflow and Harvest bottles full of media or are their vent filters wet, which could prevent removal of liquid from the flowpath?

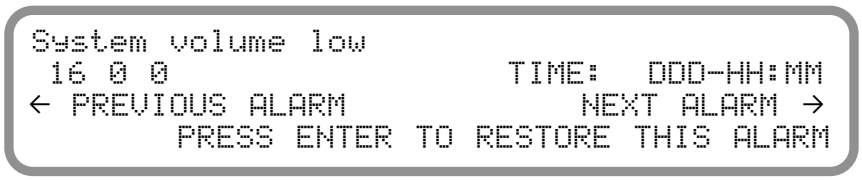


Figure 7-10: Alarm Screen 16

This alarm means the Expansion Chamber is below its minimum volume.

Take immediate action!

1. If the Expansion Chamber is empty and/or the bioreactor is filled with air:
   a) Disconnect the EC gassing line from **EC** bulkhead port.
   b) On Monitor screen, if `Rise` is shown, go to step d).
   c) On Monitor screen, if `Fall` is shown, disconnect IC gassing from **IC** bulkhead port and connect it to **EC** bulkhead port.
   d) Watch Integration Chamber volume and increase Media pump speed if necessary to maintain ~1.5 inches of medium.
   e) When Expansion chamber has ~108 ml, clear the overrides and connect IC and EC gassing lines in their normal places.
   f) Check the suggestions below and if EC chamber volumes drains again, contact Biovest's Account Services.

Verify the following items to try to correct the problem:

- Any clamps incorrectly open or closed, particularly GEX Out?
- Does the flowpath have any leaks, cracks, or loose tubing connections?
- Gassing lines fully connected to IC and EC chambers and bulkhead ports?
- Gassing lines attached to the correct bulkheads: IC to IC and EC to EC?
- Medium pump segment properly loaded in the pump head? Is its pump tubing segment leaking? Is its pump tubing segment kinked inside the pump head?
- Any unintended pump overrides still active, particularly Media Pump?
- Is the Media supply empty? Its clamp closed? Its vent filter plugged? Its tubing male/femal Luer connection loose?

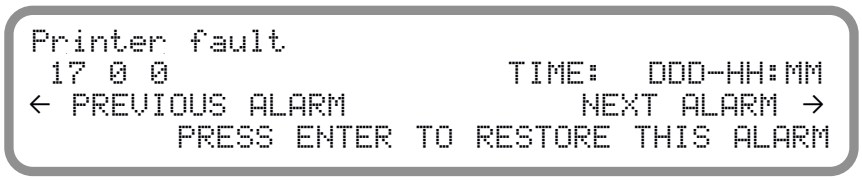


Figure 7-11: Alarm Screen 17

This alarm means the printer is unable to print for a variety of reasons. Verify the following items to try to correct the problem:

- Printer offline or deselected?
- Printer out of paper?
- Printer paper jammed?
- Printing head jammed or cannot move on its track?

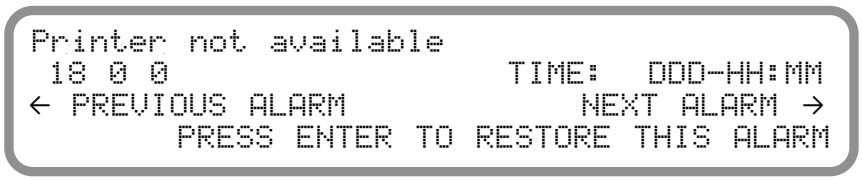


Figure 7-12: Alarm Screen 18

This alarm means the printer is not turned on, or the printer cable is not fully connected to the printer and/or instrument.

Note: **More than one printer alarm usually occurs at the same time. To minimize unnecessary printer alarms, disable the printer (SETUP screen) before adjusting the printer functions or paper supply.**

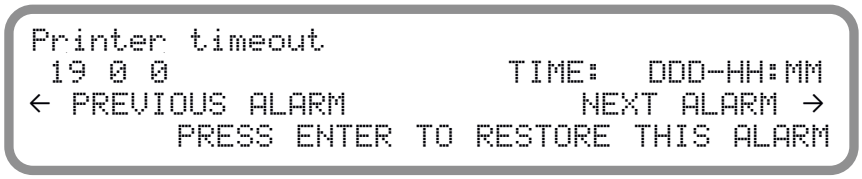


Figure 7-13: Alarm Screen 19

This alarm means attempts to send log information to the printer have failed. The printer may need service.

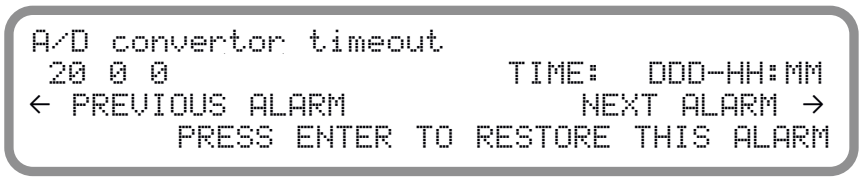


Figure 7-14: Alarm Screen 20

This alarm means the monitor board, or possibly the control board, is faulty. This problem likely requires repair service.

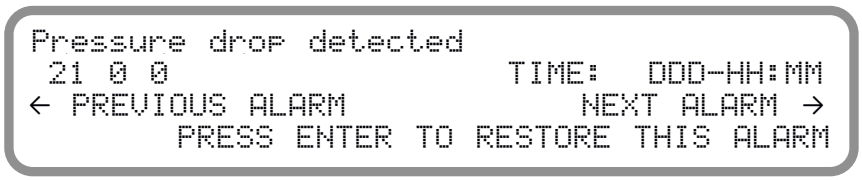


Figure 7-15: Alarm Screen 21

This alarm only occurs when the Pressurization Test fails (and not during an active culture run). It indicates there is a leak large enough to cause a rapid loss of pressure. This may happen during the pressurization test of the instrument, probe case assemblies, or cultureware.

- If EC leaked, the second number is > 0
- If IC leaked, the third number is > 0
- Verify that all connections are properly sealed on probe case assemblies
- Gassing lines are firmly connected to the IC and EC bulkhead ports and the probe case assemblies or cultureware
- If tubing extension lines or othe items were added to the cultureware, do not include them in a repeat of the Pressurization Test. If the test then passes, the integrity tubing extension lines or other items should be checked.
- Remove cable ties added to the pH and DO probe case assemblies and repeat the Pressurization Test.
- Re-check the tightness of the male Luer plugs on the cultureware and repeat the Pressurization Test.
- If the instrument Pressurization Test fails, the instrument likely needs repair service.

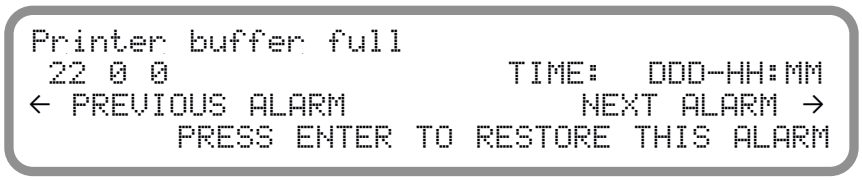


Figure 7-16: Alarm Screen 22

This alarm means the instrument's user interface buffer is unable to send characters to the printer. Verify the following possible causes:

- Printer power on?
- Printer online or selected?
- Printer paper empty?
- Another reason the printer is offline?
- Printer cable fully connected to the printer and *MAXIMIZER*?
- If the alarm persists, call Account Services.

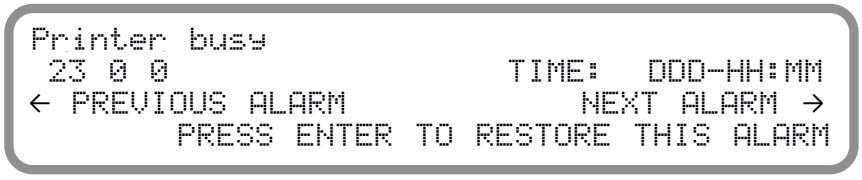


Figure 7-17: Alarm Screen 23

This alarm means the printer is unable to receive information while it is in the process of printing. Verify the following possible causes:

- Printer power on?
- Printer online or selected?
- Printer paper empty?
- Another reason the printer is offline?
- Printer cable fully connected to the printer and *MAXIMIZER*?
- Printing head jammed or cannot move on its track?
- If the alarm persists, call Account Services.

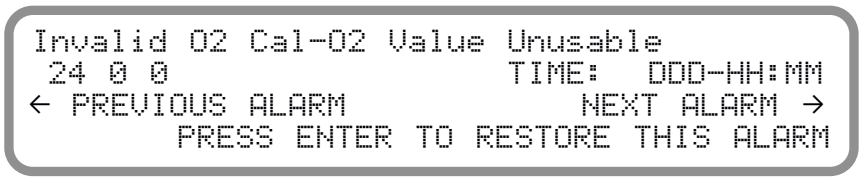


Figure 7-18: Alarm Screen 24

This alarm means the calibration value entered (data2) was zero, or the sampled A/D count (data1) equaled zero. Ensure DO calibration is properly performed.

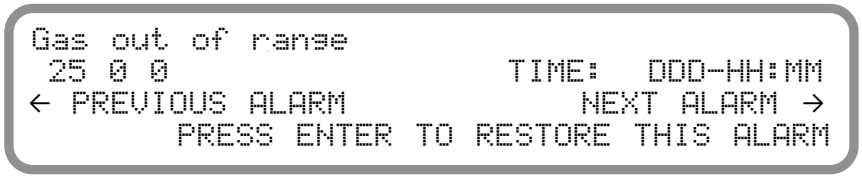


Figure 7-19: Alarm Screen 25

This alarm means the gas mixture number (1–14) is outside of the gas limits set in Process Control. Verify the following items to try to correct the problem:

- pH probe measuring accurately? If so, you may have to change the gas limit setting in Process Control.
- CO_2 gas supply properly set to deliver 15 ± 1 psi?
- Gas override active and setting the gas number outside the range?
- Calibration of the pH probe fixes problem?
- Calibration procedure being properly performed?

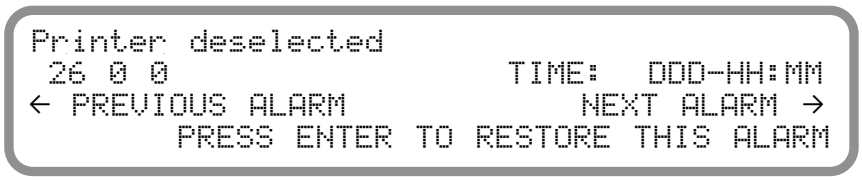


Figure 7-20: Alarm Screen 26

This alarm means the printer is offline. Ensure printer is selected or on-line. Check for "paper out" light on printer and jammed printer head.

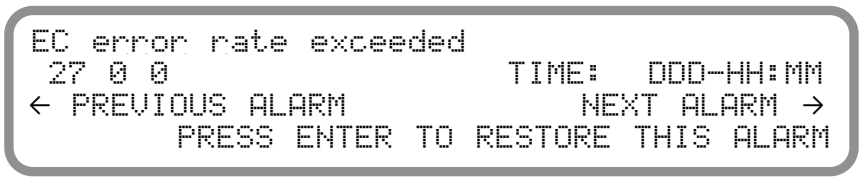


Figure 7-21: Alarm Screen 27

This alarm means the software is unable to measure a high percentage of valid readings from the ultrasonics cable.

If this alarm occurs infrequently, it may be of little concern because it could be caused by sudden vibration against the instrument (moving the instrument, slamming the door) or by shaking the cultureware tray during cell removal or other tasks.

If this alarm occurs frequently, verify the items below to try to correct the problem. It is possible the chamber could drain or flood! Watch the Expansion Chamber for a few Rise and Fall cycles to ensure its volume is properly controlled and remains between the Low (~108 ml) and the High volume (~108 ml + Transfer Volume).

- Cultureware tray in a level position in the incubator?
- Repositioning cultureware tray resolves problem?
- Ensure nothing is under cultureware tray
- Cell debris or foam in the Expansion Chamber? Performing Cell Removal Procedure resolves problem?
- Ultrasonics cable fully connected to its bulkhead jack?

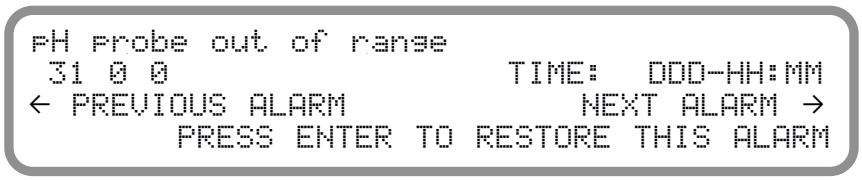


Figure 7-22: Alarm Screen 31

This alarm means the calibration procedure measured too large a difference between the ideal pH probe value and the offline value. Verify the following items to try to correct the problem:

- Calibration of the pH probe fixes problem?
- Calibration procedure being properly performed?
- pH cable fully connected to the probe and its bulkhead jack?
- pH cable faulty?
- pH probe A/D reading within acceptable range? (See Section 6, **pH Probe Evaluation**.)

Note: **Because pH control is critical to the viability of a culture, this alarm is extremely important. If you are unable to resolve the problem, control pH manually by using Gas # overrides, periodically checking pH offline, and adjusting Gas # as necessary. Otherwise, replace the pH cable or pH probe or contact Account Services.**

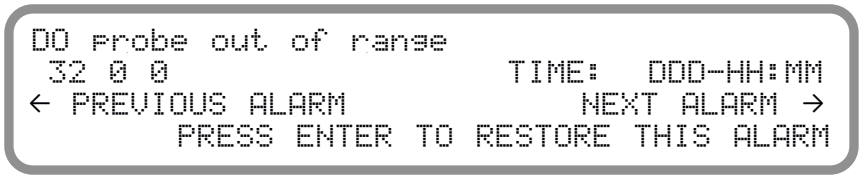


Figure 7-23: Alarm Screen 32

This alarm means the calibration procedure measured too large a difference between the ideal DO probe value and the offline value. Verify the following items to try to correct the problem:

- Calibration of the DO probe fixes problem?
- Calibration procedure being properly performed?
- DO cable fully connected to the probe and its bulkhead jack?
- DO probe A/D reading within acceptable range (See Section 6, **DO Probe Evaluation**.)? If not, you must decide whether or not to replace the probe. Contact Account Services for further details.

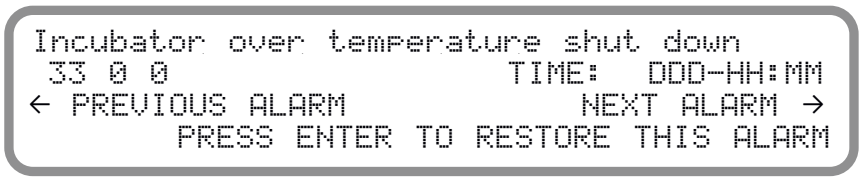


Figure 7-24: Alarm Screen 33

This alarm means the air temperature at the internal heater box has reached 65°C. This condition activates the over-temperature relay to shut off the heater, but its fan continues running to try to cool the system. The Monitor screen will show '>' to the right of the temperature value until the heater box (incubator) temperature cools down. The heater re-starts only after the heater box temperature falls to 45°C. If the ambient temperature is within specified values (Appendix A) and the problem occurs, the instrument likely requires repair service.

Note: **The incubator temperature mentioned above is measured internally with a separate temperature sensor. It should not be confused with the media (flowpath) temperature that is measured with the cable connected to the incubator bulkhead jack.**

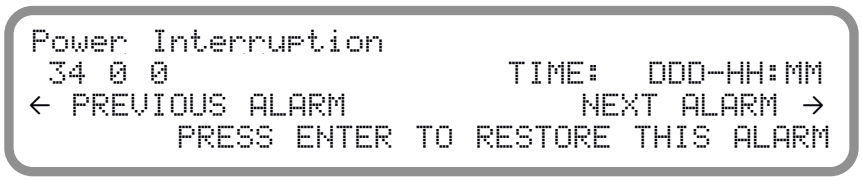


Figure 7-25: Alarm Screen 34

This alarm means the instrument has experienced a power outage, whether intentional or unintentional.

Data1 shows the number of hours, and data2 shows the number of minutes that the instrument was without power.

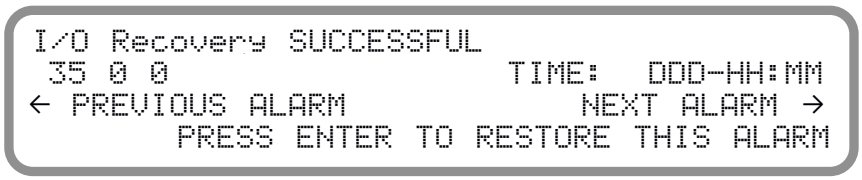


Figure 7-26: Alarm Screen 35

This alarm means the main program could not communicate with one of the secondary programs that was then followed by an electronic reset that did restore communications. Data1 indicates which processor caused the error.

If this alarm occurs very infrequently, it may not be symptomatic of a problem. If it occurs more frequently than that, the instrument should be serviced.

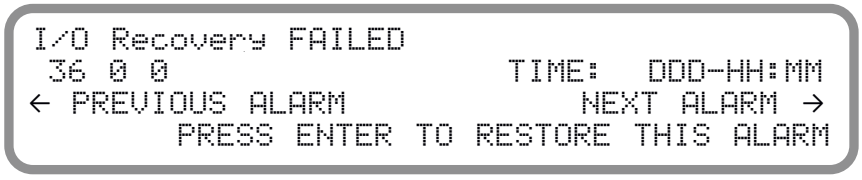


Figure 7-27: Alarm Screen 36

This alarm means the main program could not communicate with one of the secondary programs that was then followed by an electronic reset that **did not** restore communications. Data1 indicates which processor caused the error.

If this alarm occurs very infrequently, it may not be symptomatic of a problem. If it occurs more frequently than that, the instrument should be serviced.

DIAGNOSTICS SCREENS

Some of the uses of these four screens are explained in Section 5 and 6. Although these screens were meant for service personnel to check on the internal system status, you may be asked to refer to certain information during discussions with Account Services.

REMINDER: To view this series of screens, press **START**, then the hidden key (empty space between the **HOLD** key and the ⬇). Advance through the series by pressing **ENTER**.

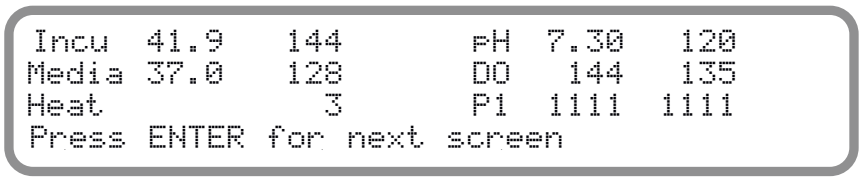


Figure 7-28: Diagnostics Screen 0

The screen shown in Figure 7-28 is called Screen 0, and you have been taught how to use the pH and DO fields to validate probes (Section 6). The fields represent the following information:

Incu Indicates the temperature of the air as it leaves the heater box. Normal operating temperature is 39–55°C. Range of A/D reading (second column) is 176–250.

Media Indicates the temperature of the media measured by the cultureware probe. At 37°C the A/D reading (second column) is 147.

Heat This indicates the number of 50 msec increments that the incubator heating element is ON during a heater cycle time of 250 msec. Completely OFF is shown as 0, while completely ON is shown as 5.

pH The first column displays the pH, while the second column displays the A/D reading from the pH probe.

DO The first column displays the DO, while the second column displays the A/D reading from the DO probe.

P1 This is a collection of bits indicating various status values on the monitor board. These are used for internal system communication only.

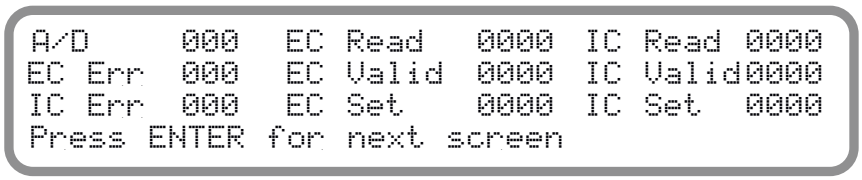

A/D 000 EC Read 0000 IC Read 0000
EC Err 000 EC Valid 0000 IC Valid0000
IC Err 000 EC Set 0000 IC Set 0000
Press ENTER for next screen

Figure 7-29: Diagnostics Screen 1

The screen shown in Figure 7-29 is called Screen 1. The fields represent the following information:

A/D This indicates the A/D time out count and should always be zero.

EC Read These are the counts from the ultrasonics. Normal volume in the Expansion Chamber during cycling (with default settings) is 108 – 178 ml. The corresponding counts are 64 – 96.

IC Read Disregard

EC Err This value indicates the number of faulty ultrasonic readings in a 30 second period and should always be less than 120.

IC Err Disregard

EC Valid This value indicates the weighted valid reading from the ultrasonics. If the ultrasonics are functioning properly, the EC Read and EC Valid values will be within 2 A/D readings of each other.

IC Valid Disregard

EC Set This value indicates the last pressure setting that was calculated for the Expansion Chamber during cycling operation. At 100 mmHg the A/D reading should be 33.

IC Set Disregard

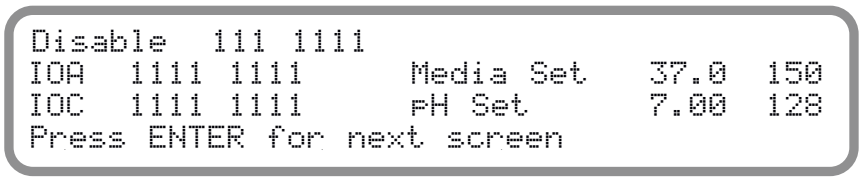


Figure 7-30: Diagnostics Screen 2

This screen is called Screen 2. The fields represent the following information:

Disable This is a collection of bits indicating whether gas, temperature, and EC control are enabled. These are for internal system communication only.

IOA These are a collection of bits that control the gas solenoids. bit0 = CO_2, bit1 = high air, bit2 = medium air, bit3 = low air (where 0 = closed, 1 = open).

Media Set These indicate the media temperature setpoint and its corresponding A/D reading. A medium temperature of 37.0° should have an A/D reading of 150.

IOC These are a collection of bits that control the chamber pressurization and venting valves. bit0 = IC gas solenoid, bit1 = IC vent solenoid, bit2 = EC gas solenoid, bit3 = EC vent solenoid (where 0 = closed, 1 = open).

pH Set This indicates the pH setpoint and its corresponding A/D reading. A pH value of 7.00 at 37°C should have an A/D reading of 128.

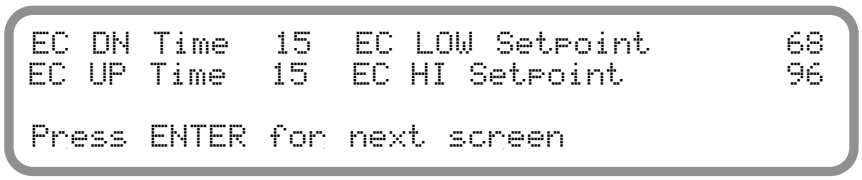


Figure 7-31: Diagnostics Screen 3

This screen is called Screen 3. The fields represent the following information:

EC DN Time This is the time in minutes that the cycling control routine will attempt to move EC fluid from the high setpoint to the low setpoint. Default value = 15.

EC UP Time This is the time in minutes that the cycling control routine will attempt to move EC fluid from the low setpoint to the high setpoint. Default value = 15.

EC LOW and EC HIGH Setpoint

These are the A/D readings where EC cycling toggles from a Rise phase to a Fall phase. The default values are 68 for LOW Setpoint and 96 for HIGH Setpoint. They correspond to Expansion Chamber volumes of 108 ml and 178 ml, respectively.

PROBLEMS WITH BIOLOGICAL PERFORMANCE

pH Control

Because pH is controlled by several factors, you may have to consider many aspects of the culture to resolve problems with pH control. The following is a list of major areas to examine. Contact Account Services for more details on pH control.

- CO_2 gas supply properly set to deliver 15 ± 1 psi?
- Gas override active and setting the gas number outside the range?
- GEX Out clamp closed?
- Correct amount of bicarbonate added to the medium?
- Medium should not include HEPES.
- pH probe functioning properly? (See Section 6, **pH Probe Evaluation**.) Also, has the pH probe been calibrated regularly?
- If a base solution is attached to Factor 1, does its addition to the culture cause changes in pH that are too great or too small? If so, you may need to replace this solution with one that has a lower or higher concentration.
- pH cable attached to the probe and bulkhead securely?
- pH probe may require preventative maintenance. Refer to the manufacturer's instructions that are provided with the probe.

Product Secretion

Failure of a culture to attain a desired level of productivity may be caused by a variety of factors. The following is a list of suggestions to consider if you find yourself in this situation. Due to the many unknowns in cell culture, it is not a comprehensive list, but Account Services personnel are prepared to discuss any problem you may have.

- Could there be problems with the product assay?
- Are there problems with storage of harvested medium? Temperature, length of storage, and pH should be considered.
- Drastic changes in productivity suggest a significant change has occurred in some aspect of process control. Changing the harvest rate or serum concentration too severely are common sources of this type of problem. Replacing serum-supplemented medium with serum-free (or similar) medium too suddenly is another.
- Changes in medium or serum lots might affect productivity due to differences in quality. You may need to examine the medium preparation process and/or quality control procedures.
- Changes in productivity may be seen immediately after cells are removed from the Expansion Chamber. The protocol used in this procedure may have to be altered.
- Harvest strategy may have to be changed to accommodate the nature of the cells' production process. For example, batch harvesting may need to be compared to continuous harvesting. In addition, some metabolic parameter (pH, DO) may need to be changed to enhance production.

Contamination

Sources of contamination: Termination of a culture run due to contamination is always a humbling experience. It is important to recognize the most likely source(s) of such a problem because it (they) must be avoided in future runs. Sometimes diagnosing the cause of contamination is simplified by observing whether the problem is restricted to the ICS or ECS loop. Refer to any records of autoclave cycles, validation of hoods and autoclaves, sterility tests, medium and serum lots, quality control procedures and any timetable that shows when sampling or bottle replacement is performed. Be honest about the history of aseptic techniques used throughout the run.

Cultureware and instrument cleanup: Remove the flowpath as described on page 6-90. Save the probe cables and gassing lines, and thoroughly clean the pH and oxygen probe case assemblies. Replace the oxygen electrode membrane, and add fresh electrolyte. The components of the probe holders can be soaked in sodium hydroxide solution (0.1N) for 30 minutes to remove endotoxin residue, then rinsed **thoroughly** in distilled water before they are washed with tissue culture detergent.

Properly dispose of the contents of all bottles that were attached to the flowpath. Bottles and cap assemblies may be treated like the probe holders with sodium hydroxide solution. Vent filters for cap assemblies should be discarded as a biohazard.

Disinfect the incubator, the outer surfaces of the *MAXIMIZER* cabinet and the interior of the door. Avoid spilling any solutions on the circulation pump rotor, in the bulkhead connections and in the incubator exhaust vent.

HARDWARE AND CULTUREWARE

Pumps

To ensure optimum performance, the peristaltic pumps should be cleaned after each culture run. Media spills and small pieces of worn pump tubing may be trapped inside the plastic housing and should be removed. Wipe the interior of each plastic shell with a cloth or paper towel soaked in disinfectant or alcohol. Wipe the surface of the metal rollers with a cloth or paper towel soaked in alcohol. Allow any moisture to evaporate before you re-assemble the pump head. Each pump head requires two white, teflon washers. Replacements must be installed before the next run. See Appendix B, **Ordering Information**.

Note: **Do not soak the pump head or rollers in alcohol because it will strip away the necessary lubricant.**

Cultureware

Each cultureware assembly is assigned a lot number which may be used to trace defects specific to a certain build. This number is located on the left corner of the tray (near the Outflow tubing). In addition, the shipping box for the cultureware has a manufacturing number which identifies the sterilization lot. These numbers should be recorded and used when any cultureware problem is brought to the attention of Account Services.

Appendix A - Specifications

ACUSYST-*MAXIMIZER*® SPECIFICATIONS

Note: **FOR INDOOR USE ONLY**

Instrument Dimensions

Width:	69.8 cm (27.5 inches)
Depth:	66.0 cm (26.0 inches)
Height:	52.0 cm (20.5 inches) [door closed]
	73.6 cm (29.0 inches) [door open]

Instrument Weight

54.5 kg (120 lb)

Operating Temperature

External (Ambient):	18 – 26°C (64–79°F)
Internal (Incubator:	24.1 – 45.8°C (75–114°F)

Electrical Requirements

P/N: 600052-000:	120 VAC, 4 amp, 60 Hz, 500 watts
P/N: 600052-001:	100 VAC, 4 amp, 50 or 60 Hz, 500 watts
P/N: 600052-002:	230 VAC, 2 amp, 50 or 60 Hz, 500 watts

CAUTION: **Supply voltage fluctuation not to exceed ± 10% of nominal voltage (Refer to Appendix B)**

Note: **Biovest International's printers require 120 VAC and 0.7 amps.**

Backup Power Requirements (Instrument and Printer)

Uninterruptible Power Supply (UPS), 500 watts, sine wave form

Main Fuse

120VAC:	4 Amp 3AG Time Delay (SLO-BLO), ¼ x 1¼ inch
100VAC:	4 Amp 3AG Time Delay (SLO-BLO), ¼ x 1¼ inch
230VAC:	2 Amp Type T (SLO-BLO, need 2), Metric 5 x 20 mm

Appendix A - Specifications

Heater Box Fuse

(Not intended for replacement by Operator)
1.5 Amp 3AG Time Delay (SLO-BLO), ¼ x 1¼ inch

Power Supply Fuse

(Not intended for replacement by Operator)
1.5 Amp 3AG Time Delay (SLO-BLO), ¼ x 1¼ inch (230VAC units only)

Pollution Degree

II

Installation Category

II

Battery Type

Sanyo Lithium CR-2N, 3 Volt (M_nO_2) (Serviced by authorized personnel only)

Gas Requirements

Necessary:	100% Carbon Dioxide (medical grade), 15 psi (1 kg/cm^2)
Backup (optional):	100% Air (medical grade), 15 psi (1 kg/cm^2)
As Needed:	Outgas (biohazardous applications only), slight vacuum

Cultureware and Accessories

Disposable, pre-sterilized:	Flowpath with Pharmed pump tubing, bioreactor gas exchanger, silicone tubing, Tygon tubing, ultrasonics
Reusable, non-sterile:	Electrode (pH, DO, temperature), probe cases, bottles, cap assemblies, vent filters, Tygon gassing lines

Approximate Flowpath Volumes

600058-215:	IC + EC Circuit Volume = 570 ml EC Circuit Volume = 230 ml (at low vol.) EC Circuit Volume = 300 ml (at high vol. *)
600058-221:	IC + EC Circuit Volume = 615 ml EC Circuit Volume = 275 ml (EC low vol.) EC Circuit Volume = 345 ml (EC high vol. *)
600068-215:	IC + EC Circuit Volume = 680 ml EC Circuit Volume = 340 ml (at low vol.) EC Circuit Volume = 480 ml (at high vol. **)
600068-221:	IC + EC Circuit Volume = 770 ml EC Circuit Volume = 430 ml (EC low vol.) EC Circuit Volume = 570 ml (EC high vol. **)

* 70 ml transfer volume
**140 ml transfer volume

Appendix A - Specifications

Peristaltic Pump Rate Ranges

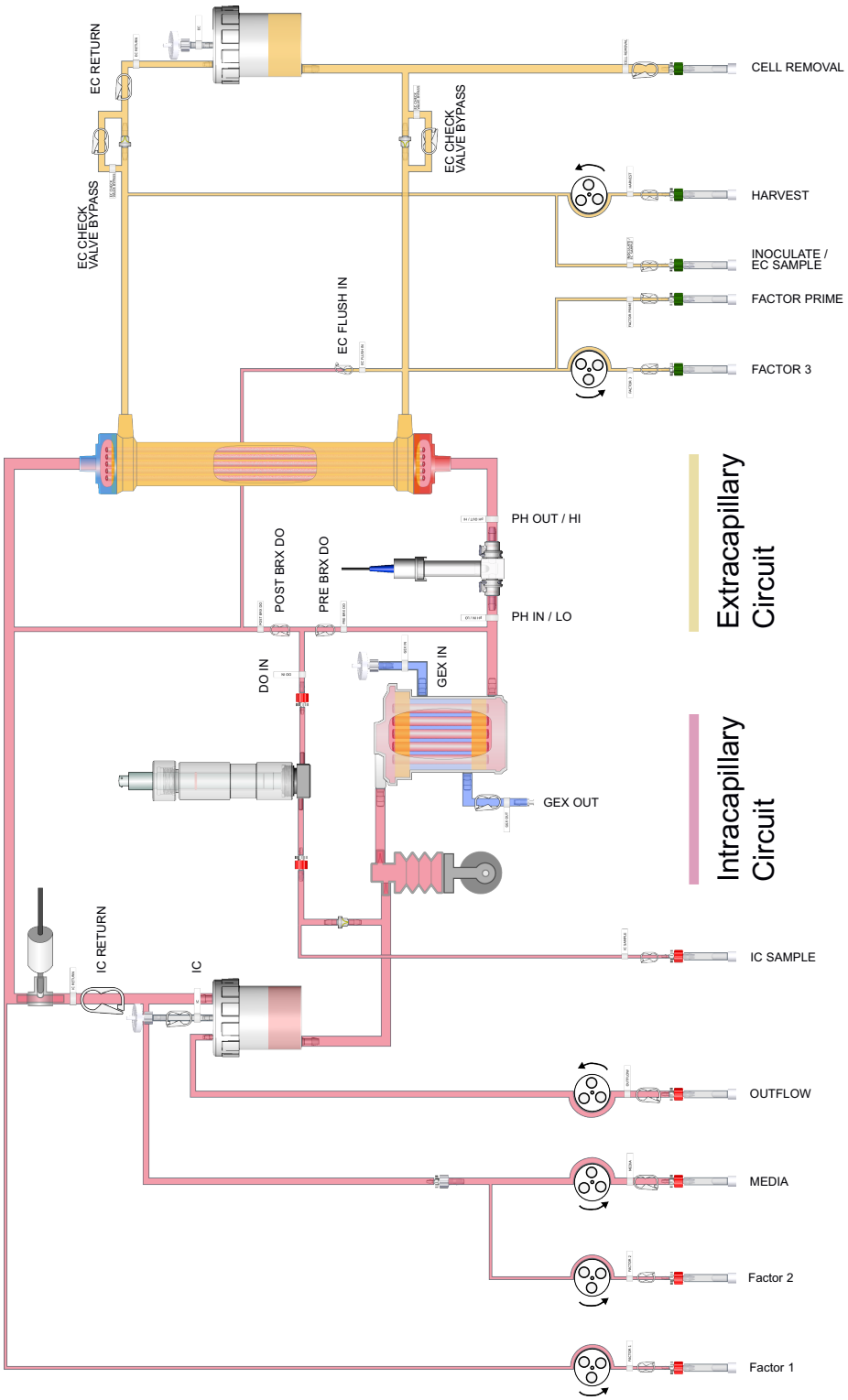
	Range		Increment
Process Control Pump Rates:			
7016 Pump Heads			
Outflow Pump	0– 4320	ml/hr*	1 ml/hr
Media Pump	0 – 800	ml/hr	1 ml/hr
7014 Pump Heads			
Factor 1 Pump	0 – 200	ml/hr*	1 ml/hr
Factor 2 Pump	0 – 200	ml/hr	1 ml/hr
Factor 3 Pump	0 – 200	ml/hr	1 ml/hr
Harvest Pump	0 – 200	ml/hr	1 ml/hr
* Automated—no direct user input			
Manual Override Pump Rates:			
7016 Pump Heads			
Outflow Pump	0 – 4320	ml/hr	1 ml/hr
Media Pump	0 – 800	ml/hr	1 ml/hr
7014 Pump Heads			
Factor 1 Pump	0 – 400	ml/hr	1 ml/hr
Factor 2 Pump	0 – 400	ml/hr	1 ml/hr
Factor 3 Pump	0 – 400	ml/hr	1 ml/hr
Harvest Pump	0 – 400	ml/hr	1 ml/hr
Fill/Flush Media Pump Rate:	4320	ml/hr, automated	
Inoculation Outflow Pump Rate:	1000	ml/hr, automated	
Circulation Pump Rate Range	0 – 1000	ml/min	1 ml/min

Note: Same range and increment for process control and manual override.

CAUTION: Maximum user-entered CP speed when using a single bioreactor cultureware must be ≤ 500 ml/min to prevent excessive pressure, tubing disconnection, severe media leak, and contamination. Using CP speeds > 500 ml/min will void the cultureware's warranty!

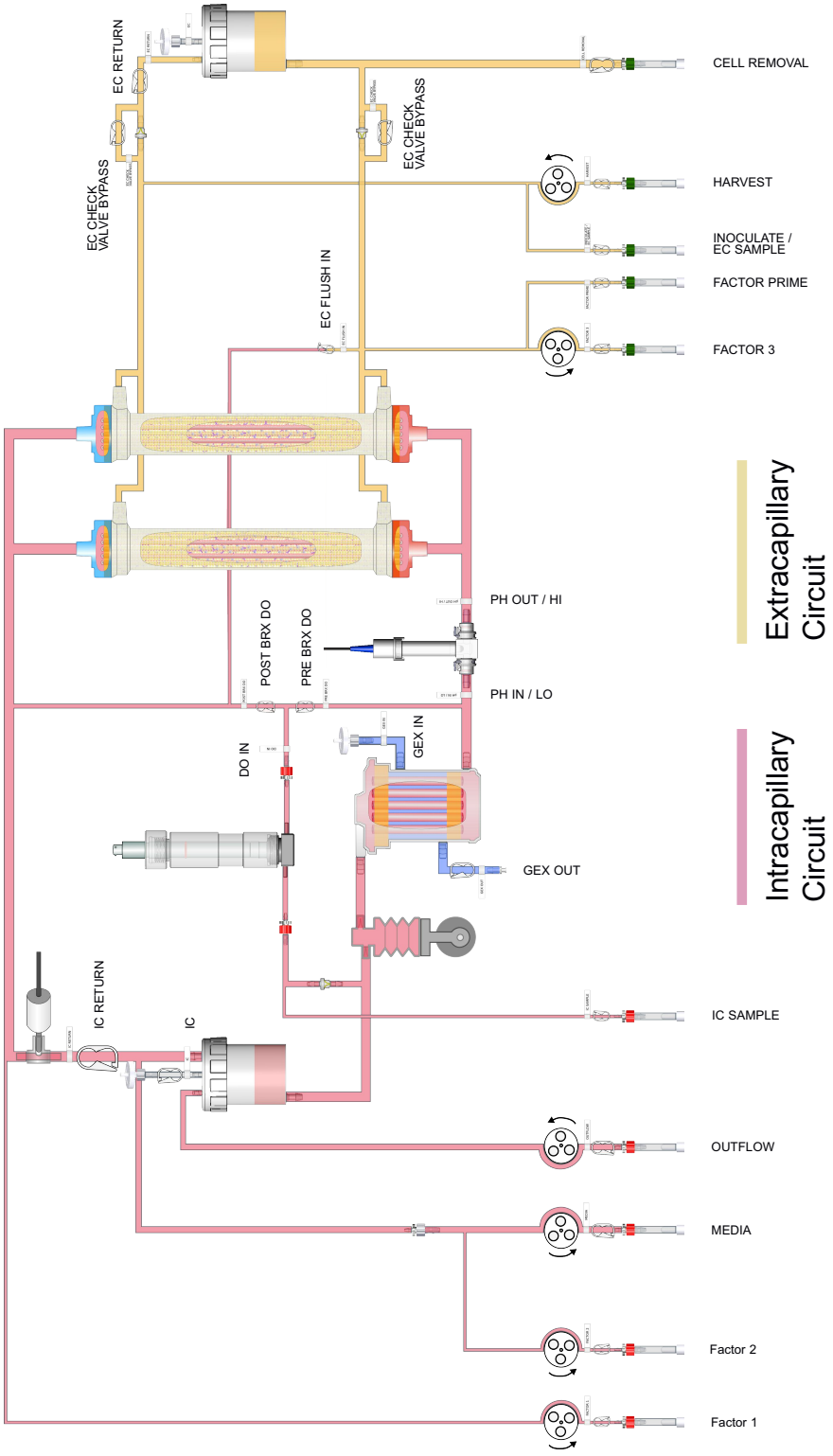
Note: All pumps rotate counterclockwise only.

Schematic for 600058-xxx Cultureware Models



Appendix A - Specifications

Schematic for 600068-xxx Cultureware Models



Appendix B - Ordering Information

These orderirng part numbers represent common items needed to operate the AcuSyst-*Maximizer*. Other miscellaneous items also are available, so if you need something not on the list, please contact Biovest's Account Services group.

Part Number	Description Of Part
Instrument and Accessories	
600052-XXX	AcuSyst-*Maximizer*® Cell Culture System -000 (120 VAC) -001 (100 VAC) -002 (230 VAC)
4190-000	Printer Package (100/120 VAC)
600072-000	AcuData™ Software Package
600095-000	AcuLink-Data Log™ Base Unit
600096-000	AcuLink-Data Log™ Complete (100/120 VAC)
600096-001	AcuLink-Data Log™ Complete (230 VAC)
Cultureware Options	
600058-215	Single 1.5 m^2 Bioreactor Flowpath
600058-221	Single 2.1 m^2 Bioreactor Flowpath
600068-215	Double 1.5 m^2 Bioreactor Flowpath
600068-221	Double 2.1 m^2 Bioreactor Flowpath
Sterile pH Probe Items	
600305-000	pH Probe, Sterile, Ready-for-Use, Single-Use
600306-000	pH Probe Holder, Reusable (use with 600305-000 only)
Reusable pH Probe Items	
103037-000	pH Electrode, Non-sterile, Reusable
103047-000	pH Electrode Storage Solution, 250 mL
401090-000	pH Electrode Cable
600298-000	pH Electrode Holder
600300-000	pH probe Tubing Lines (spare set of two)
600301-000	pH Electrode Holder O-rings, (spare set of three)
Dissolved Oxygen Probe Items	
4192-001	Oxygen Electrode Re-membrane Kit (replaces 4192-000)
102323-000	Oxygen Electrode Electrolyte, 25 mL
3923-000	Oxygen Electrode Cable
5330-000	Oxygen Electrode Holder O-ring (spare, qty. 1)

Appendix B - Ordering Information

400831-000	Oxygen Electrode & Electrolyte
600057-000	Oxygen Electrode Holder
600054-000	DO Probe Tubing Lines (spare set of two)

Miscellaneous Sterile Optional Items

600063-000	In-line Media filters, Small (4, Individually packaged)
600063-001	In-line Media filters, Large (4, Individually packaged)
600100-072	1/16″ Sterile Tubing Extension, six feet long
600101-072	1/8″ Sterile Tubing Extension, six feet long Both extension lines are silicone and include: • Male luer to connect to the cultureware • Male and female luers to connect to the container • Pinch clamp at the container connection end

600318-000	Luer-Activated Valve (LAV) Kit, 15 each of: • Sterile and Individually Packaged LAVs • Polypropylene Luer Dust Covers (Sterilizable)

Reusable Glass Bottles and Polypropylene Cap Assemblies

Bottles are borosilicate with polypropylene pouring ring, GL45 thread and volume markings. Caps are polypropylene with two liquid ports (accept male luer slip connection), one vent port (1825-000 filter purchased separately), and they include internal silicone tubing to deliver or collect fluid. Each part number is a package of four items.

600013-000	Media Bottle, 0.5 liter
600014-000	Media Bottle, 1.0 liter
600015-000	Media Bottle, 2.0 liters
600016-000	Media Bottle, 5.0 liters
600017-000	Media Bottle, 10.0 liters
600018-000	Delivery Cap Assembly, 0.5 liter
600019-000	Delivery Cap Assembly, 1.0 liter
600020-000	Delivery Cap Assembly, 2.0 liters
600021-000	Delivery Cap Assembly, 5.0 liters
600022-000	Delivery Cap Assembly, 10.0 liters
600023-000	Receiving Cap Assembly, use on any volume bottle
600061-000	Bottle & Cap Startup Package, includes: • Six 1-liter bottles • Four 10-liter bottles • Two 10-liter delivery cap assemblies • Four 1-liter delivery cap assemblies

- Four receiving cap assemblies (fit any size bottle)
- One box of vent filters (see 1825-000)

Cap Assembly for Plastic Bottles

Cap is polypropylene size 53B with two liquid ports (accept male or female luer lock connections) and one vent port (filter included). It is fully assembled, including all necessary external and internal tubing, clamps and color-coded labels to deliver or collect fluid. It is ready-for-use. User only needs to autoclave-sterilize it.

400836-000	Delivery and Receiving Cap Assembly, (use with 2L Nalge bottle)

Miscellaneous Spare Parts

1825-000	Sterilizable, Reusable Vent Filters (50 per box)
3033-000	White Sterility Protector Vent Plug (each, minimum 10)
3429-027	4 Amp Fuse (100/120 VAC units)
3675-019	2 Amp Fuse (230 VAC units)
4197-000	Size 14 Pump Head
4198-000	Size 16 Pump Head
100201-000	Urethane Pump Head Coupler
102064-000	Pump Head White Teflon Washers (pack of 12)
400136-000	Operations Manual
400137-240	Circulation Temperature Probe
400148-000	Circulation Pump Hardware • Metal Screws (two) • Plastic Threaded Knob (one)
600156-000	Incubator Gassing Lines, one set: • GEX IN, GEX OUT, IC, EC

Services

CALIB	Calibration (recommend annually)
SERV	Installation Qualification, Calibration, Operation Qual.
SERV CON	Service Contract (renewable, one-year coverage)
400828-000	Preventive Maintenance (recommend every five years)

Appendix C - Relocation

If you need to move the ACUSYST-*MAXIMIZER* to another room or building, you should consider the following recommendations before starting any cultures in the instrument.

Preparing the *MAXIMIZER*

- Turn off the power to the *MAXIMIZER* before disconnecting the power cord from the wall outlet.
- Remove any plastic cable ties from the CO_2 gas line on the rear of the instrument before disconnecting the tubing. Then, remove the tubing carefully to avoid breaking the plastic fitting on the filter. Allow ample work space to do this efficiently.
- Be certain you have the cables for temperature probe, pH probe, and DO probe, as well as the four Tygon gassing lines.
- The gas filters on the rear of the *MAXIMIZER* do not have to be removed when the instrument is moved. However, use caution to avoid damaging the filters or their mounting brackets when you place the instrument on a cart and when you move through narrow doorways. This also applies to the peristaltic pumps on the front of the instrument.
- Be certain you have the plastic screw cap and the 2 metal screws for the circulation pump bracket.

Appendix C - Relocation

Instrument Lifting Instructions

The Instrument is quite heavy. Two people working together should be used to lift and carry the instrument. Failure to follow these instructions could result in injury to the person(s) lifting or carrying the instrument or damage to the instrument. **Handle with care. Do not** lift the instrument by grasping the door or gas filters. Grip sides and back of instrument only.

After the move ...

- Have the instrument's internal system serviced by a qualified technician. This will ensure that transporting the *MAXIMIZER* has not damaged electrical or mechanical components.
- Be certain the electrical circuit in the new room will not be overloaded by the installation of the *MAXIMIZER*, and that it contains the desired backup power facility.

Note: **Contact Biovest International's Account Services Department for additional instructions if relocation requires shipping the MAXIMIZER over extended distances. Biovest International recommends shipping the MAXIMIZER in specially designed packaging only.**

Appendix D - Metabolic Calculations

This section details calculations to determine uptake rates for glucose, glutamine, and oxygen, and the production rates of lactate, ammonia, and secreted product. The following information applies to all metabolic calculations:

$e' = e^{-rt/v}$

where: e = 2.7183
r = total inflow rate (ml/h)
t = t2 - t1 (rounded to the nearest integer)
t1 = time of previous sample
t2 = time of current sample
v = system volume:
Single bioreactor 1.5 m^2 Flowpath = 570 ml
Double bioreactor 1.5 m^2 Flowpath = 615 ml

medium rate = (medium $volume_{t2}$ - medium $volume_{t1}$) ÷ t
factor rate = (factor $volume_{t2}$ - factor $volume_{t1}$) ÷ t
glucose rate = (glucose $volume_{t2}$ - glucose $volume_{t1}$) ÷ t
serum rate = (serum $volume_{t2}$ - serum $volume_{t1}$) ÷ t
base rate = (base $volume_{t2}$ - base $volume_{t1}$) ÷ t
harvest rate = (harvest $volume_{t2}$ - harvest $volume_{t1}$) ÷ t
r = medium rate + factor rate + glucose rate + serum rate + base rate

Appendix D - Metabolic Calculations

The remainder of this section describes individual calculations for rates of cellular metabolism and productivity.

GLUCOSE UPTAKE

The calculation of glucose uptake is based on the following equation:

$$GUR = \frac{Glucose_{in} - r\ Glucose_{t2} - e'(Glucose_{in} - r\ Glucose_{tl})}{1 - e'}$$

where:

GUR	=	Glucose Uptake Rate (mg/h)
$Glucose_{t2}$	=	current glucose reading (mg/ml)
$Glucose_{t1}$	=	previous glucose reading (mg/ml)
Gm	=	conc of glucose in medium (mg/ml)
Gg	=	conc of glucose in glucose supplement (mg/ml)
Gs	=	conc of glucose in serum (mg/ml)
Gf	=	conc of glucose in factor (mg/ml)
$glucose_{in}$	=	(medium rate * Gm) + (glucose rate * Gg) + (factor rate * Gf) + (serum rate * Gs)

LACTATE PRODUCTION

The calculation of lactate production is based on the following equation:

$$LPR = \frac{r\ (Lactate_{t2} - e'\ Lactate_{tl})}{1 - e'}$$

where:

LPR	=	Lactate Production Rate (mg/h)
$Lactate_{t1}$	=	previous lactate reading (mg/ml)
$Lactate_{t2}$	=	current lactate reading (mg/ml)

GLUTAMINE UPTAKE

The calculation of glutamine uptake is based on the following equation:

$$GltUR = \frac{Glut_{in} - r\ Glucose_{t2} - e'(Glut_{in} - r\ Glut_{tl})}{1 - e'}$$

where:

GltUR	=	Glutamine Uptake Rate (mmol/h)
Gltm	=	glutamine concentration in medium (mmol/ml)
Gltf	=	glutamine concentration in factor (mmol/ml)
$Glutamine_{in}$	=	(medium rate * Gltm) + (factor rate * Gltf) (mmol/h)
$Glutamine_{t1}$	=	previous glutamine reading (mmol/ml)
$Glutamine_{t2}$	=	current glutamine reading (mmol/ml)

AMMONIA PRODUCTION

The calculation of ammonia production is based on the following equation:

$$APR = \frac{r\ (Amm_{t2} - e'Amm_{tl})}{1 - e'}$$

where:

APR	=	Ammonia Production Rate (mmol/h)
Amm_{t1}	=	previous ammonia reading (mmol/ml)
Amm_{t2}	=	current ammonia reading (mmol/ml)

Appendix D - Metabolic Calculations

OXYGEN UPTAKE

Some investigators prefer to use the oxygen update, or consumption rate (OUR) as a measure of a culture's metabolic status. The calculation of OUR is based on the following equation:

$$OUR = \frac{CP\ (pO2_{out} - pO2_{in})}{k}$$

where:

OUR = Oxygen Uptake Rate (mmol/hr)

$pO2_{(out)}$ = post-BRX DO (mmHg)
$pO2_{(in)}$ = pre-BRX DO (mmHg)
CP = circulation pump rate (ml/min)
k = oxygen solubility constant = 13,333 $^{ml \cdot mmHg \cdot hr}/_{mmol \cdot min}$

PRODUCT FORMATION

The calculation of product formation is based on the following equation:

$$PFR = 24 \times f \times Product$$

where:

PFR = Product Formation Rate (units/day)
f = Harvest pump flow rate (ml/h)
Product = current product concentration (units/ml)

Appendix E - Loading Pump Heads

The following instructions show the proper technique to load tubing into the peristatic pump heads. These techniques achieve the most consistent accuracy and precision in actual pumping flow rates relative to the desired pumps speeds entered in Process Control. This consistency applies to all of the six pumps during a single production run, as well as, to these pumps for numerous production runs over time.

These techniques also result in the **liquid flowing through the pump heads in the correct direction!** Be especially careful when reviewing figure E-3!

1. Hold the pump in your left hand, the side with the slot in the roller assembly facing up (Figure E-1).

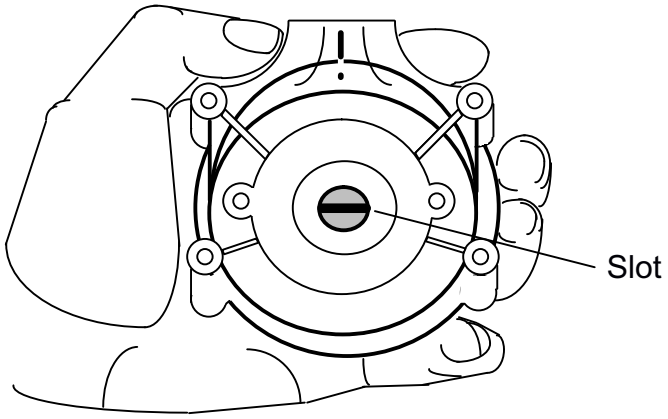


Figure E-1: Front of peristaltic pump head

2. Remove the front half of the pump head.
3. Remove the roller assembly and examine both sides (Figure E-2). Assure that the Teflon washers, one per axle, are clean and in-place. Replace the roller.

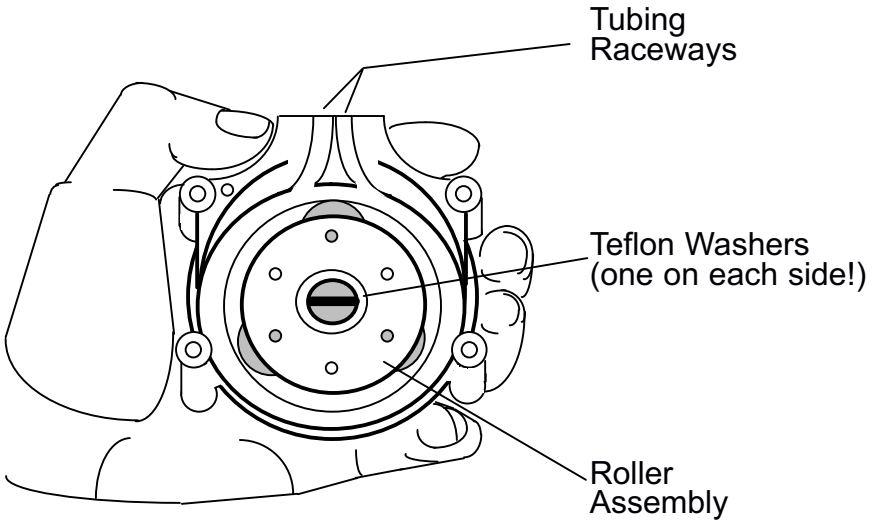


Figure E-2: Pump head roller assembly

4. With your right hand, position the pump segment as shown in Figure E-3. At this point shift the pump segment counterclockwise from how you ultimately want it when loaded because the loading process shifts the pump segment clockwise.

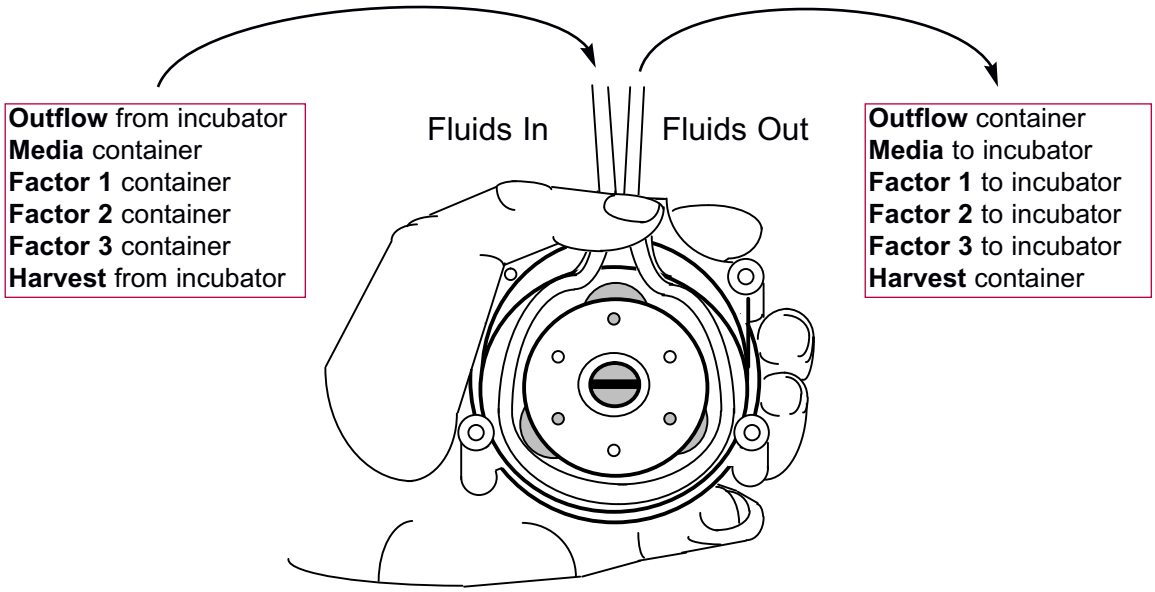


Figure E-3: Tubing placement

Note: **The orientation of each pump segment must be considered. Fluids enter the pump head on the LEFT and exit on the RIGHT because all peristaltic pumps on all ACUSYST systems operate counterclockwise. Outflow and harvest are loaded in one orientation (to pump these fluids out) while all other pumps are loaded in the opposite orientation (to pump these fluids in).**

Note: **Wrap the tubing around the roller by following its existing curvature. Tubing loaded against its existing curvature will have a tendency to twist resulting in greater inaccuracies in flow rates and premature wear of the pump tubing segment.**

5. With your left thumb, securely hold the tubing in the right raceway. Be sure that your hand is positioned as shown in the figures. Correct hand position is essential to the successful completion of the procedure.
6. With your right hand, slide the pump key into the position as shown, so that it presses down on the tubing (Figure E-4).

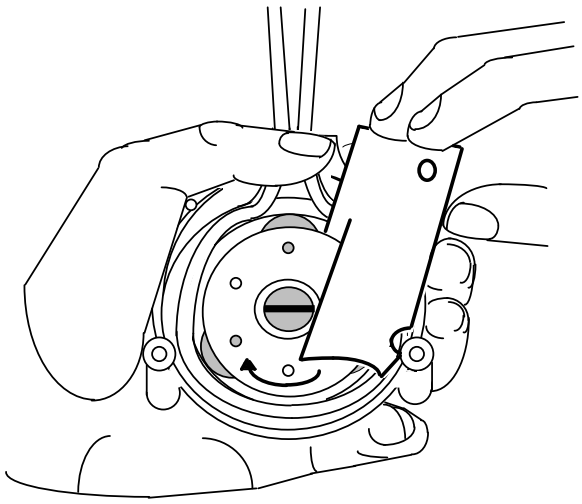


Figure E-4. Pump key position

7. Pushing the roller down with the key, turn the pump key clockwise. The key presses approximately half of the tubing's diameter in the pump casing. Turn key until approximately the ten o'clock position, where there is a stop tab.

NOTE: **Hold the left pump segment loosely while you turn the pump key. As the pump key positions the tubing in place, the left pump segment needs to be able to slide under your left thumb.**

8. Remove the pump key. Hold the pump tubing firmly in both raceways with your left thumb as you also place the top half of the pump over the roller.
9. Push the two halves of the pump head together. The halves will 'snap' together and the edges will be flush when they are properly connected (Figure E-5). If you are concerned that the two halves did not go together as expected, take the pump head apart, remove the tubing, and start again at step 4.

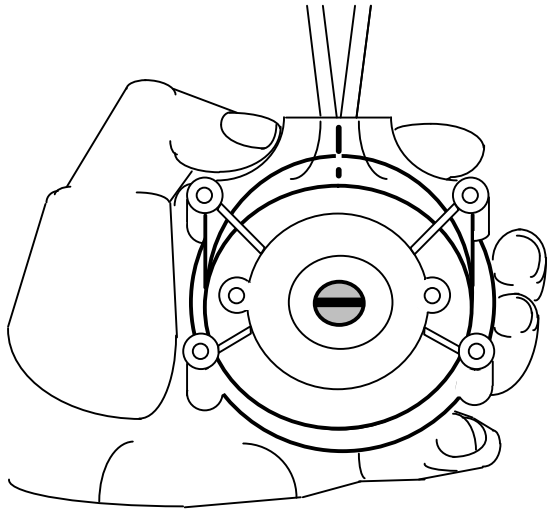


Figure E-5: Completed pump head assembly

CAUTION: DO NOT pull, stretch, or otherwise manipulate the tubing inside the pump in an effort to get the two pump head halves together in step 9. The tubing will not return to its normal state after extended operation and the result will be decreased flow rate and variances between pumps.

PROBE STORAGE

pH Probe

The reusable pH probe should be stored with its electrical cap attached and it's tip in pH storage solution, see Ordering Information in Appendix B. A storage bottle is provided with the probe to hold the storage soluttion.

Store new disposable pH probes in the same manner as for cultureware.

DO Probe

The DO probe should be stored with its electrical cap attached and the plastic cap and sponge covering its membrane tip. Store the probe vertically with the tip downward. Leave the electrolyte in the membrane cartridge if the probe will be used in the next ~6 months. If storage time will be longer, store the probe without electrolyte in the membrane cartridge.

The DO probe requires replacement parts to work properly over many years of service. The replacement parts are provided in a Re-membrane Kit, see *Ordering Information* in Appendix B. Usage information is in *DO Probe Evaluation* in chapter 6, *Operating Procedures*.

Other considerations for pH and DO probes:

- Aggressive cleaning of the reusable pH probe and the DO probe is generally not necessary. Rinsing these probes with water at the end of the production run is usually sufficient.
- Store them at room temperature.
- Do not store them in a room with a high relative humidity.

Appendix F - Maintenance

PUMP HEADS

Pump heads routinely accumulate small pieces of tubing debris during production runs. This debris must be cleaned at the end of each production run.

Pump heads require two white teflon washers. They wear out over time. Replace them if they are worn or missing, see *Ordering Information* in Appendix B.

Pump heads typically last for many years of service. However, if the metal roller shows signs of wear or doesn't revolve freely in the pump head, replace the pump head, see *Ordering Information* in Appendix B.

CLEANING

Use a gentle detergent solution to clean the cabinet. Do NOT spray liquid on the electrical connections and keypad (and cabinet openings!) to prevent liquid from entering into the cabinet.

Clean any cell culture medium spill in the incubator. Use caution to ensure medium — or any other liquid — does NOT enter the cabinet via the opening for the circulation pump motor!

ACUSYST® INSTRUMENT SERVICE

Biovest offers the following validation and defined maintenance services to facilitate the use of your ACUSYST system in a Quality System Regulation environment. These services also help to keep its operation as trouble-free as possible and to minimize unplanned downtime and disruptions to manufacturing operations.

Installation Qualification / Operational Qualification

IQ/OQ validation service is optional with a new instrument purchase. Validation can be performed before the instrument's first use, at a later time, and whenever it is relocated. IQ/OQ includes Calibration.

Calibration

Biovest International specifies annual calibration of all ACUSYST systems. Adherence to the schedule facilitates consistent, optimal manufacturing. Calibration returns the instrument's electrical and mechanical systems to Biovest's factory standards.

Preventive Maintenance

Biovest International specifies a five-year interval for preventive maintenance on all ACUSYST systems. The purpose of preventive maintenance is to minimize the risk that the system's operation will fail and prematurely end a production run, which could further delay future production scheduling. Preventive maintenance replaces all wearable components of the system and includes calibration.

Full Service Contract

Biovest International offers a renewable, annual service contract to ensure the instrument is maintained in normal working order by covering all parts, labor, and an amount of travel expense for the contract year.

Glossary

ACUSYST	**A**utomated **C**ulture **S**ystem.
Bioreactor	Polycarbonate case containing thousands of hollow fibers where media is exchanged and a cell culture grows.
BRX	See **Bioreactor**.
Bulkhead	Connection panel located near the top inside of the incubator.
Cap Assembly	Biovest International's patented vented screw-cap for glass bottles. May be used for feed or receiving bottles.
Circulation Pump	Bellows pump which circulates media throughout the IC flowpath.
Clamp	Device which restricts flow through tubing.
Cultureware	The disposable and non-disposable components of the flowpath and its associated sensors, cables, and tubing.
Expansion Chamber	The fluid reservoir in the Expansion Circuit.
EC	See **Expansion Circuit**.
EC Cycling	Ultrafiltrative movement of media through the bioreactor fibers from the IC to the EC. One cycle consists of an EC Rise and an EC Fall.
ECS	See **Extracapillary Space**.
Expansion Circuit (EC)	The portion of the flowpath that includes the outside of the fibers in the bioreactors, the Expansion Chamber and the associated tubing.
Extracapillary Space (ECS)	The space in the bioreactor outside of the hollow fibers where cells are grown (part of the Expansion Circuit). Can also be used to refer to the volume in the bioreactor where the cells are grown.
Factor	A supplement, or addition, to a growth medium. This may be serum, insulin, transferrin, or other components typically added to the ECS or ICS.

Glossary

Flowpath	All components of the EC and IC; see **Cultureware**.
Gas Exchange Cartridge	The cartridge in the cultureware which is responsible for the introduction of gases (CO_2 and O_2) into the media. Also known as an oxygenator.
GEX	See **Gas Exchange Cartridge**.
Harvest	The process of collecting media containing cell product. or The media which contains the cell product.
Hollow Fibers	The thousands of porous hydrophilic tubes within a bioreactor. They permit exchange of nutrients and wastes and serve as growth substrate for cultured cells.
IC	See **Integration Circuit**.
ICS	See **Intracapillary Space**.
Integration Chamber	The media reservoir for the Integration Circuit.
Integration Circuit	The portion of the flowpath which includes the inside of the fibers, the integration chamber and associated tubing. The Integration Circuit is separated from the Expansion Circuit by the walls of the hollow fibers.
Intracapillary Space (ICS)	The volume inside of the fibers of the bioreactor.
LCD	Liquid crystal display at the top of the control panel. Used to show all of the user interface screens.
Medium Dilution	The process of removing high levels of waste or raising the pH by increasing the medium flow rate.
MWCO	Molecular weight cutoff, measured in daltons. Refers to the pore size of the hollow fibers.
Outflow	Effluent from the IC, media containing metabolic by-products which are small enough to pass through the pores of the fibers.

Oxygenator	See **Gas Exchange Cartridge**.
Peristaltic Pump	The metal and plastic pumps which move fluid into and out of the flowpath for media, factor, harvest, and outflow.
Pre-sample	The portion of the media that is removed before the sample to be analyzed. The pre-sample contains media which may not be representative of that which is in the culture.
Pump Head	The plastic portion of the peristaltic pump which encloses the pump segment of the tubing.
Sample	The media removed from the flowpath for metabolic or chemical analysis.
Sample Port	A controlled opening in the flowpath which permits removal of media for analysis. A sample port may end in a female Luer-Lok fitting or a septum.
Setpoint	The value of a given metabolic parameter (pH, temperature) which is to be maintained by the software.
Tubing Raceway	A grooved plastic panel used for routing tubing into the incubator and refrigerator.
Vent Filters	Either the filters used for introducing gas into the flowpath (to the Integration and Expansion Chambers) or the hydrophobic 0.2 micron pore filter (Acrodisc) on the cap assemblies which prevent pressure buildup.